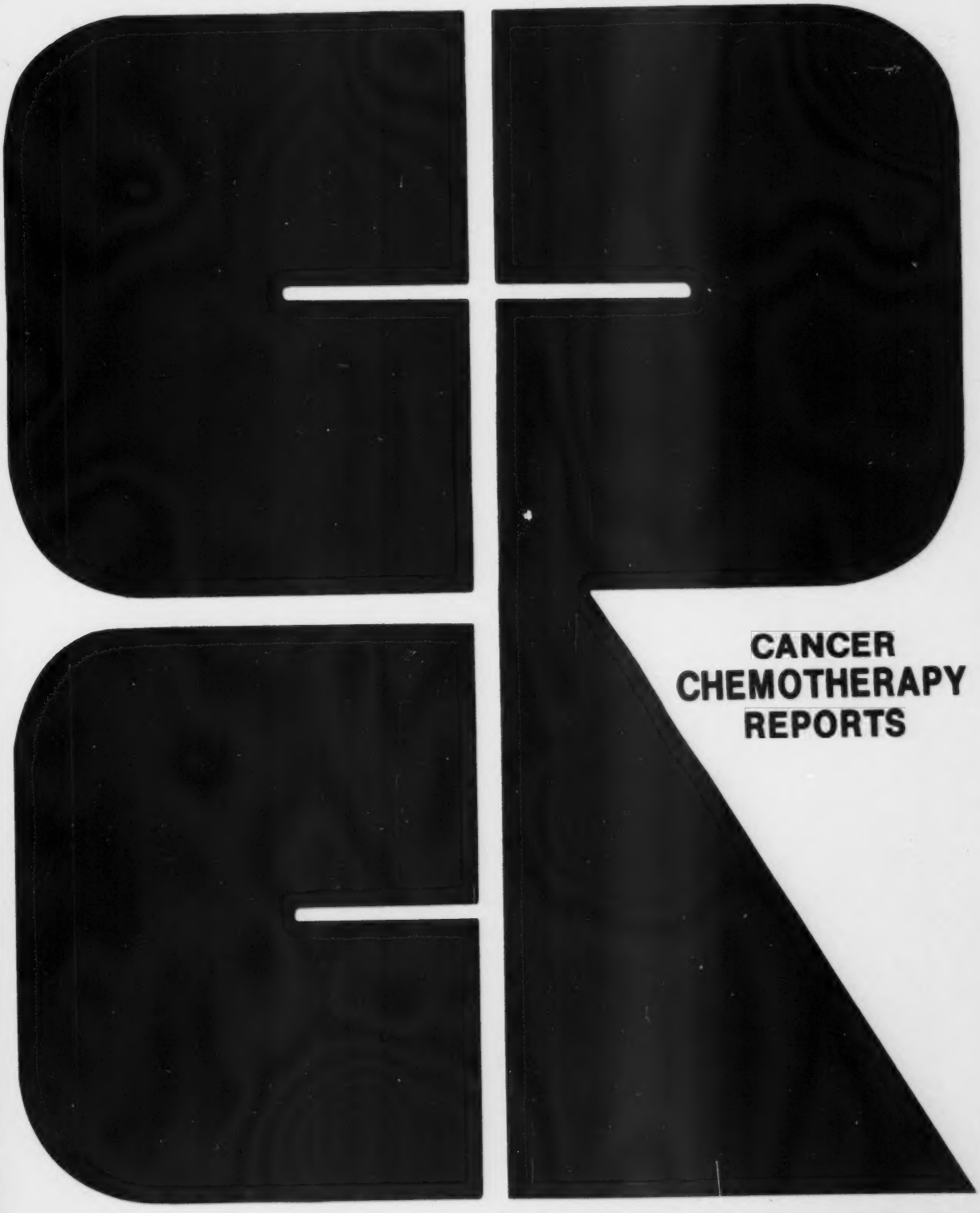

# CANCER CHEMOTHERAPY REPORTS

Part 1

Nov/Dec 1975
Vol. 59, No. 6

# Cancer Chemotherapy Reports


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Begin the article with a short summary.

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## ANNOUNCEMENT

This is the last issue of *Cancer Chemotherapy Reports*. The next issue of this journal will be entitled *Cancer Treatment Reports*. The new title reflects the fact that the journal is sponsored by the Division of Cancer Treatment, National Cancer Institute. Its scope will now encompass all aspects of cancer treatment including chemotherapy. The editors hope that this change will focus attention on the totality of cancer treatment which increasingly involves a multimodality approach. In the future contributions will therefore be solicited and encouraged from investigators involved in studies on all aspects of the treatment of cancer.

In addition to a change in title and scope, the journal will be altering its system of volume numbers. Issues will no longer be referred to as Part 1, 2, or 3. A total of 12 issues (in one volume) will be published each year, with consecutive page numbers and issue numbers. In 1976, issues will be identified as volume 60, number 1 (January), volume 60, number 2 (February), etc. Each year six issues will be devoted to reviewed original research manuscripts and six issues will contain the proceedings of meetings and symposia or nonreviewed program information. We hope that this simplified system will be more understandable and useful to our readers.

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67th Annual Meeting of the American Association for Cancer Research, Inc
Hematology/Oncology Fellowship
Symposium on Recent Advances in Cancer Treatment Research

*The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.*

# LETTERS

## Cardiotoxicity of 5-Fluorouracil (NSC-19893)[1]

Many reports have recently appeared on the cardiotoxicity of adriamycin (*see Cancer Chemotherapy Reports* Part 3, vol 6, No. 2, 1975). We wish to report similar toxic effects in man after the use of 5-fluorouracil.

5-Fluorouracil has been used as a cytostatic agent in oncologic practice since 1957 (1). Today it is predominantly used in the treatment of patients with adenocarcinoma of the digestive tract and carcinoma of the breast and ovary, as well as for patients with soft tissue sarcoma.

The toxic effects of the drug have been described by many investigators (2-6) and can best be divided into gastrointestinal complaints, myelosuppression, dermatologic changes, and neurotoxic effects. Presently, there are no reports on the toxic effects of 5-fluorouracil on the myocardium, especially on blood vessels with resulting stenocardias. Recently, the accumulation of labeled 5-fluorouracil (5-fluorouracil-2-$C^{14}$) in the myocardium of experimental

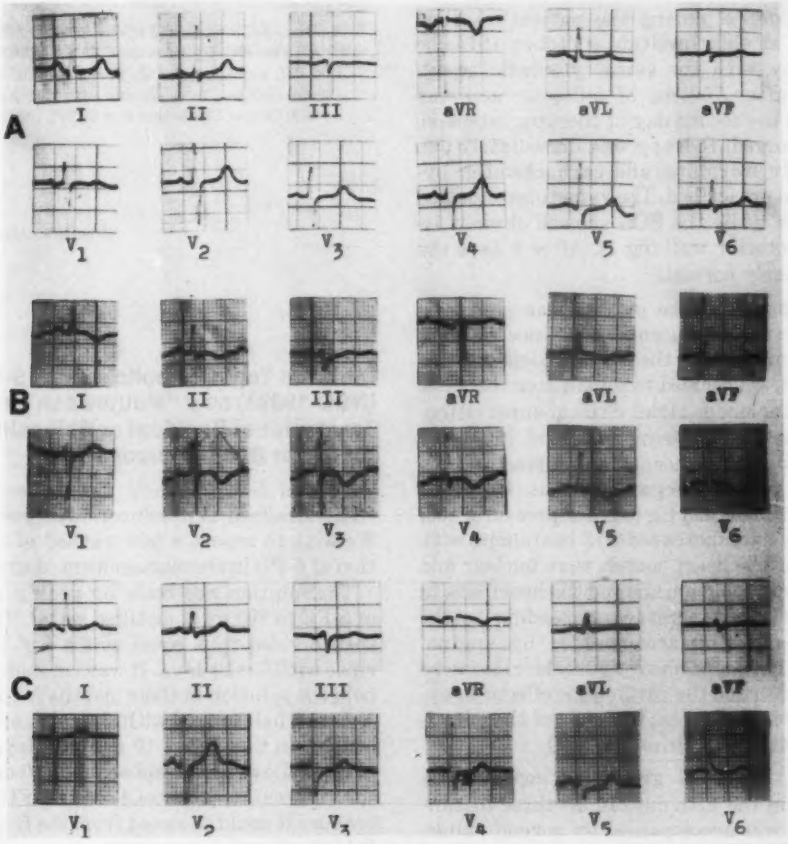


FIGURE 1.—A = ECG before treatment with 5-fluorouracil; B = ECG 4 hrs after iv administration of 750 mg of 5-fluorouracil; C = ECG 5 days later (shows complete reversibility of the cardiologic changes). The same ECG changes were recorded during the first and third instances of iv administration of 5-fluorouracil.

[1]5-Fluorouracil: CAS reg. No. 51-21-8.

animals has been observed, but toxicity was not observed (7). We therefore consider it instructive to report on one of our patients (a 48-year-old man) who experienced this toxic reaction.

A complete clinical evaluation resulted in a diagnosis of adenocarcinoma of the rectum with suspected metastases to the liver. We began chemotherapy with daily doses of 750 mg of 5-fluorouracil. After the second day of this therapy, the patient complained of mild precordial pain which became more pronounced on the fourth day when the electrocardiogram (ECG) indicated ischemic changes in the anterior wall. When the cytostatic therapy was discontinued, the ECG became normal and chest pains disappeared. The patient had received a total of 4500 mg of 5-fluorouracil. Since this event was considered a coincidence, during the patient's second hospitalization at this Institute (October 1974) he received therapy with the same cytostatic agent. Several hours after 750 mg of 5-fluorouracil was administered on the second day of therapy, intensive stenocardia occurred; therapy was immediately discontinued and iv morphine and carbochromen hydrochloride were prescribed. The symptoms subsided after 15 minutes while the ECG showed obvious ischemia of the anterior wall (fig 1). After 3 days the ECG was completely normal.

We may mention that the patient was not given any drugs other than 5-fluorouracil. Since we were unable to find any data on the cardiotoxicity of this cytostatic agent, we decided to administer the same drug 10 days later under strict clinical supervision. Four hours after the iv administration of 750 mg of 5-fluorouracil, serious stenocardia occurred and the patient became pale and began to sweat. His blood pressure fell to 125/80 mm Hg (earlier pressure, 150/90) and the pulse rate increased (115 beats/min) with rhythmic action. The heart sounds were unclear and muffled. The roentgenogram showed the heart size to be normal. The pains increased, spreading to the neck and left arm and accompanied by angina. Morphine and carbochromen hydrochloride were administered again and the cardiologic effects disappeared after several minutes. Ischemia of the anterior wall was registered again on the ECG.

Subsequently, the ECG gradually improved to become normal on the fifth day. In all three instances, stenocardia was accompanied by normal values of the serum enzymes (creatine phosphokinase, lactic dehydrogenase, and serum glutamic oxaloacetic transaminase). The patient was discharged to home care with the recommendation that he not use 5-fluorouracil again. Three weeks after his discharge, the patient had no cardiologic complaints and the ECG was normal.

We therefore feel it advisable that a regular check be made of the ECGs of patients who are receiving this cytostatic agent, and particularly for those who experience even the least precordial sensations during therapy.

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July 11, 1975


## Intraoral Topical Application of 5-Fluorouracil (NSC-19893) as a "Mouthwash" in the Treatment of Residual or Relapsing Cancer of the Lip or Buccal Mucosa[1]

Topical 5-fluorouracil (5-FU) has been used to treat localized skin cancer with good response (1). We wish to report a new method of topical application of 5-FU in the management of oral cancer.

The solution was made by adding 1000 mg (20 ml) of 5-FU to 100 ml of distilled water. The solution was then divided into equal parts for the patients to rinse with for 10 days. It was recommended that they hold the solution in their mouths for 10 minutes/day. A cotton ball soaked with the solution was applied to lesions on the lip for 10 minutes/day. A 1-week rest period followed each subsequent 10-day course. This solution was used instead of the 5-FU cream (Efudix) because it could be saved from the fresh portion of 5-FU left after iv treatment.

Five patients received from two to ten courses of this treatment. All patients had residual or localized relapsing cancer of the lip, buccal mucosa, or soft palate and could no longer be treated by surgery or

[1]5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU.

radiotherapy. Almost complete disappearance of all tumor masses occurred within 2 weeks in two patients, with a duration of response of over 27 and 107 days. In one patient < 50% tumor regression of buccal mucosa and lip lesions was achieved for 1 month, although the lip lesions progressed further later. In this patient relapsing disease at the buccal mucosa started to regress within 24 hours after the first treatment. Two patients had progressive disease.

There was no evidence of systemic toxicity from 5-FU. The lowest granulocyte count was 2703 cells/$mm^3$. Two patients developed mild stomatitis for a few days but it was not necessarily due to the drug.

Since the new topical 5-FU application as a "mouthwash" is simple and has low toxicity, it may be another method of topical cancer chemotherapy for the management of localized residual or relapsing cancer of the oral cavity and lip. Further studies to determine the proper dosage and schedule and its true response rate are indicated.

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August 25, 1975

## COMMENTARY

# Laminar Air Flow Room Reverse Isolation and Microbial Suppression to Prevent Infection in Patients With Cancer[1]

**Stephen C. Schimpff**[2,3]

Infectious complications are often of considerable concern in planning antineoplastic drug therapy. Recently, many programs to prevent infection have been designed so that the underlying advanced cancer can be more adequately treated. This commentary will attempt to (*a*) review the background logic for reverse isolation, air filtration, and microbial suppression as methods of infection prevention, (*b*) summarize the existing clinical data in these areas, and (*c*) suggest some new avenues for the future.

The incidence and severity of infection have been especially marked in acute nonlymphocytic leukemia (ANLL); death from infection has frequently interrupted the initial attempts at remission induction. Because these patients would not only benefit from infection prevention but would also provide a sensitive measure of prevention efficacy, they have been the experimental subjects of most studies of protected environments.

### Origin of Infection in ANLL

The majority of infections in ANLL during the first weeks of therapy usually occur in only five or six sites and are caused by an equal number of organisms (1-6). Pneumonia, perianal-rectal lesions, pharyngitis, skin lesions, hepatitis, and urinary tract infections account for most infections and are usually caused by gram-negative bacilli (notably *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli), Staphylococcus aureus,* the agents of viral hepatitis, and *Candida* species. Other sites (eg, esophagus) and other organisms (eg, *Aspergillus* species) are not usually important until later in the patient's course.

Designing methods of infection prevention depends first on an appreciation of the predisposing factors contributing to the majority of infections. The profound and prolonged granulocytopenia which accompanies induction therapy (<1000 granulocytes/mm$^3$ for approximately 50% of induction time) is the most significant factor predisposing to infection (7). Other contributing factors include mucosal alterations resulting from disease and therapy, indwelling catheters (both urinary and venous), other invasive procedures, contaminated intravenous (iv) solutions and transfusions, and inadequate patient hygiene (2,6).

Recent evidence indicates that most infections are caused not by the patient's endogenous flora but rather by organisms which are acquired in the hospital and colonize in the patient awaiting an opportune moment to infect (1). Organisms such as *P. aeruginosa,* for example, reach the patient through food, water, and inadequate personal hygiene and then colonize in the orointestinal tract. Later, a break in mucosal or integument integrity of the anus, bowel, pharynx, esophagus, or skin, or stasis of fluids due to inadequate tracheobronchial clearance or urethral obstruction occurring in the presence of granulocytopenia allows the rapid development of infection with bacteremia commonly resulting (6,8,9).

As with *P. aeruginosa* infection, available data also suggest that *S. aureus, Aspergillus* species, and probably many cases of *K. pneumoniae, E. coli, Serratia* species, and *Enterobacter* species infections are frequently if not usually caused by hospital-acquired strains and not by strains which were part of the patient's original endogenous flora (1).

The above considerations suggest that infection prevention depends upon (*a*) bolstering the patient's host defense mechanisms (vaccines, cell support programs, and, ultimately, complete remission of leukemia), (*b*) reducing invasive procedures (urinary and venous catheters) while caring for natural barriers (mucosa and skin), (*c*) preventing hospital acquisition of potential pathogens (reverse isolation), and (*d*) suppressing the microbial flora present both in and on the patient (oral nonabsorbable antibiotics and topical antibiotics and antiseptics).

### Bolstering Host Defenses and Reducing Invasive Procedures

Bolstering host defenses and reducing invasive procedures must be basic to the care of all patients with ANLL. Certain killed vaccines (influenza) are

[1]Received June 14, 1975; accepted July 23, 1975.

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[3]*Reprint requests to:* Stephen C. Schimpff, MD, BCRC, University of Maryland Hospital, 22 S Greene St, Baltimore, Md 21201.

safe and effective; vaccines for *P. aeruginosa*, other gram-negative bacilli, and hepatitis B are under investigation (10-12). Passive immunization against hepatitis B (immune serum globulin) and varicella (zoster immune globulin) are possibly effective if given immediately after exposure (13,14). The possibility of HL-A matched granulocytes as infection prophylaxis has yet to be critically investigated, whereas prophylactic platelet transfusions may possibly assist in infection control by preventing hemorrhage.

Although iv catheters are convenient, their use cannot be justified in patients with leukemia except during shock or rapid massive blood product administration. The alternative use of butterfly needles, changed every 48 hours, will substantially reduce the incidence of bacteremia in these patients. Urinary catheters likewise have no place in the management of leukemia except for urinary obstruction or during shock. In the absence of catheters, urinary tract infections are generally limited to patients with obstructive lesions or a past history of recurrent urinary infection (2,6).

Skin infections are common, especially in intertriginous regions (axilla, perineum, groin) or areas of damage such as the axilla after shaving or using occlusive antiperspirants. Assiduous attention to patient hygiene (bathing, oral care, perianal care) may substantially reduce integumentary or oral mucosal infections.

More complex techniques such as reverse isolation and/or oral nonabsorbable antibiotics, although standard in many clinics today, must be considered only as adjuncts to the above measures, measures which should be implemented in any hospital which treats patients with adult acute leukemia.

## Reverse Isolation

The importance of infections caused by hospital-acquired organisms suggests that strictly enforced reverse isolation should reduce the frequency of infections in these patients; however, no appropriately controlled investigation of reverse isolation in a standard hospital room has been published. All of the available data on the efficacy of reverse isolation derive from studies of laminar air flow (LAF) rooms, Life Island units,[4] or similar devices which combine air filtration with reverse isolation in a specially constructed room or unit.

Surprisingly, relatively little data are available on the specific usefulness of these units themselves because most investigators have also included an oral nonabsorbable antibiotic regimen for reduction of the resident microbial flora (whether it be exogenous or endogenous). However, the data available from the use of LAF units without microbial suppression strongly suggest that intensive reverse isolation alone does indeed reduce the frequency of infection in granulocytopenic patients with acute leukemia (15-19). One investigation spanning 5 years clearly indicated that granulocytopenic leukemia patients treated in LAF room isolators had only minimal inhospital acquisition of *S. aureus* and gram-negative bacilli plus no infections with organisms generally presumed to be spread by the airborne route (eg, *Aspergillus* species) (15). A prospective study comparing patients treated with isolation alone, isolation plus antibiotics, or neither found that LAF room isolation alone was able to significantly reduce infections (16). Indeed, this reduction was about equal to that of the combined modality method (LAF isolation plus nonabsorbable antibiotics). The finding that reverse isolation alone will reduce infection supports the concept that hospital-acquired organisms are a major cause of severe infection in these patients. Another prospectively controlled trial, based on data collected by a cooperative group, also found that isolation alone reduced infection, especially pneumonia (17,19). Although the available results are strongly suggestive of efficacy, more data will be necessary from further prospectively controlled trials to further define the role of reverse isolation in the absence of microbial suppression.

Equally important will be information indicating whether strictly enforced reverse isolation in a standard hospital room is as efficacious as LAF room isolation in preventing hospital acquisition of organisms and subsequent infection. Mechanically, the only obvious difference between routine reverse isolation and LAF[5] room isolation is air filtration. Although gram-negative bacilli, the most frequent causative organisms of infection, are probably food-, water-, fomite-, and hand-borne to the patient, the spores of *A. flavus* and *A. fumigatus* are inhaled. To date, no infections caused by *Aspergillus* species have been reported in a patient housed continuously from the time of admission in an LAF room or similar room with effective ultra air filtration,[6] whereas such infections are increasingly common in unprotected adult patients with acute leukemia (20).

LAF rooms define a space wherein only sterile materials are to be utilized (along with sterile water

[4]Mathews Research, Environmental General Corp, Alexandria, Va.

[5]Air flow is not truly laminar in quality, prompting some writers to use the term "unidirectional air flow units."

[6]Based on published accounts plus verbal communications with numerous investigators in the United States and Europe.

and low microbial content food), and the high efficiency particulate air (HEPA) filtration system ensures a constantly changing supply of air free of bacteria, fungi, and many viruses (21-23). Systemized cleaning procedures plus the unidirectional flow of air provide the additional presumed advantages of preventing build-up of microorganisms shed from the patient. Finally, and not to be overlooked in evaluation, the specialized appearance of the unit probably lends considerable psychologic support to the patient and the staff in their efforts to observe the requirements of reverse isolation.

## Microbial Suppression

Another major approach to infection prevention has been to suppress the patient's microbial flora with agents such as oral nonabsorbable antibiotics and topical antiseptics (16,17,19,24-31). Despite a number of careful trials, including some which had appropriate concurrent controls, the value of microbial suppression alone is equivocal. Two prospective trials concluded that oral nonabsorbable antibiotics alone were of no value in reducing infection (despite nearly doubled complete remission rates in one study) (25,26), while two other trials found considerable efficacy (24,27).

The results of our initial prospective controlled trial at the Baltimore Cancer Research Center appeared sufficiently convincing (24) that we now treat every adult acute leukemia patient with oral nonabsorbable antibiotics[7] during induction therapy. This decision was based on the observation that adults with ANLL who received a combination of high-dose oral vancomycin, gentamicin, and nystatin every 4 hours had fewer infections, fewer bacteremias, fewer fatal infections, and a delayed onset of those infections which did occur when compared to similar concurrent patients randomly allocated to not receive the oral nonabsorbable antibiotics (24). From August 1973 to March 1975, 87 additional patient trials with the oral nonabsorbable antibiotics have been conducted. The results clearly indicate the continued efficacy of this combination in reducing severe infections when utilized as part of a total program of infection control (32).

The antibiotics have distinct disadvantages, however, irrespective of their infection-prevention efficacy. First, the taste is extremely unpleasant and nausea, vomiting, and diarrhea are frequent. Second, the patient who discontinues the antibiotics while still granulocytopenic frequently becomes infected with the organisms (usually *P. aeruginosa*) that rapidly repopulate the orointestinal tract (24,32). Third, resistance to the antibiotics, especially to gentamicin, can occur (27,32,33). Finally, the cost is over $100 per day for the combination of vancomycin, gentamicin, and nystatin.[7] The oral antibiotic regimen should therefore only be utilized in a setting where understanding and cooperation exist between physicians, nurses, and pharmacists in their daily approach to the patient. It definitely should not be used as prophylaxis in the absence of an overall intensive program of infection prevention.

## Reverse Isolation and Microbial Suppression

To date, most trials have combined reverse isolation (such as LAF rooms) with microbial suppression (15-17,19,24,25,27-30,34-38). A number of controlled trials have now been completed comparing such a combined approach to routine hospital care (16, 19,24,25,27,34,35). As a group, the isolated microbially suppressed patients had significantly fewer infections of all severities. It appears that this combined approach will regularly reduce pneumonias, anorectal lesions, pharyngitis, and urinary tract infections. There has been no reduction in the incidence of hepatitis, related to contaminated blood products, or infections which arise from resident flora present at admission and residing in areas difficult to eradicate (eg, *S. aureus* in the nose) (24,25).

The question that immediately arises is whether such an intensive program is needed, that is, would reverse isolation alone (with or without air filtration and with or without a psychologically imposing environment) or oral nonabsorbable antibiotics alone be equally efficacious. Although both questions have been investigated, the results from different studies are not unanimous. Some trials suggest that combined modality therapy is considerably superior to any single aspect (25,35) and others suggest that LAF room isolation alone or microbial suppression alone is almost as efficacious (16,19,24,27). The only definitive conclusion possible at present is that further careful investigations must dissect these questions.

## Remission Induction

Although infections can be reduced in adult acute leukemia, there is no unanimity as to whether this reduction has any marked effect on the treatment of the underlying disease. Ultimately, the aim of initial antileukemic therapy is to produce a complete remission. In evaluations of isolation, or microbial suppression, or both, the complete remission rates have been unchanged in some (16,25), possibly increased

[7]Vancomycin (liquid form, 500 mg), gentamicin (liquid form, 200 mg), and nystatin ($1 \times 10^6$ units, liquid suspension; $4 \times 10^6$ units, tablets) given orally every 4 hours.

in some (19,34,35), and definitely increased in others (19,24,26,36). The divergency in results exists when one considers only the concurrently controlled trials (16,24-26,34,36). Overall, no definitive statement can be made other than the observation that if remission rates are in fact increased, then the increases are not of overwhelmingly obvious proportions (39). Nevertheless, the decrease in infectious morbidity and mortality among patients treated under conditions of careful infection prevention cannot be denied.

## Future Directions

There are other areas where reverse isolation, or microbial suppression, or both might offer useful advantages as methods of infection prevention. Tumors such as histiocytic lymphoma, embryonal rhabdomyosarcoma, or small cell carcinoma of the lung are frequently responsive to chemotherapeutic agents but the tumors tend to regrow before marrow toxicity abates. Placing such a patient in strict reverse isolation (eg, an LAF room) with microbial suppression (oral nonabsorbable antibiotics) might allow repeated courses of intensive chemotherapy over a short time span regardless of the prolonged period of induced granulocytopenia. Furthermore, if no infection occurs at a given chemotherapeutic dose level, it may be reasonable to increase the dose in the next course. Preliminary unpublished trials from a few institutions suggest that increased doses of chemotherapy can often be given safely to patients with sarcomas and non-Hodgkin's lymphoma who are housed in LAF rooms and are receiving oral nonabsorbable antibiotics, but definitive results are not yet available. A note of caution, however, has been reported for patients with ANLL who continue to receive intensive induction therapy past the time of marrow aplasia; three of four patients died of sepsis while in LAF rooms and receiving oral nonabsorbable antibiotics (39).

Bone marrow transplantation is another area in which protected environments and microbial suppression deserve careful evaluation (40). Presently, many patients die of graft versus host reactions, infection, or both. Infection, per se, might be reduced with these techniques and at least one controlled clinical trial is presently in progress. Furthermore, there are animal data which suggest that graft versus host reactions are reduced or eliminated when isolation and microbial suppression are utilized for 1-2 weeks before marrow transplantation (41).

Of current interest is the use of therapy of induction intensity for leukemia after a year or more in complete remission. Preliminary data suggest that when done under the cover of LAF room isolation with oral nonabsorbable antibiotic microbial suppression this technique is safe and may contribute to a prolonged disease-free survival (42).

In addition to new uses for present methodology there is a need to advance with new technology. LAF rooms require a large initial monetary expenditure and are relatively immobile (21,43). If air filtration per se is proven to be of clinical value (it has not as of this time), then it is possible that simpler portable units with HEPA filters at the head and foot of the bed will serve to adequately clear the air of microbes while allowing easy movement of the units from room to room as needed (44). Likewise, ultraviolet air sterilization might be equally effective (and much less complex) in reducing airborne contamination of bacteria and viruses (and possibly fungal spores). The oral nonabsorbable antibiotics presently in use are distasteful and have certain potential dangers, but no new antibiotics are on the horizon which have an adequate spectrum while producing less toxicity. Perhaps an alternative approach would be to feed patients a totally assimilated sterile diet which would produce only minimal residue and a multilogarithmic decrease in colonic bacteria and fungi (45). Unfortunately, these diets are often unpalatable.

Reaching a totally germ-free state in the human is exceedingly difficult but an alternative approach might include substituting a microbial flora of minimal virulence for the patient's resident microbes. A precedent in humans was successfully established in the past when avirulent staphylococci were allowed to colonize newborns, thus blocking colonization by pathogenic strains of *S. aureus*.

Yet another approach to infection prevention may be transplantation of autologous frozen marrow to patients with solid tumors treated with intensive antitumor chemotherapy or prophylactic use of matched granulocytes.

These are but a few of the many approaches in need of careful investigation. Of critical importance today is that each new investigation of either reverse isolation or microbial suppression be designed in such a manner that it asks specific, relevant questions and that the questions asked be answered as a result of the evaluation. The true value, potential efficacy, and real or potential disadvantages of protected environments and microbial suppression are just beginning to be recognized. Too often major commitments have been made in emotion and finances but not in scientific design with the result that valuable time and effort have been expended without further resolution of serious medical issues.

Certain questions which appear to be important have been suggested above. The conflicting values of historical controls, matched controls, or prospective

randomized controls have been much debated (46-48). Although the most effective method for selecting controls is perhaps not resolved, it is obvious that appropriately controlled trials that seek to answer fundamental questions are essential. The data available suggest that the combined use of LAF room reverse isolation and oral nonabsorbable antibiotic microbial suppression does reduce the frequency of serious infection. However, the use of LAF room isolators alone or microbial suppression alone by oncology centers in the absence of an organized experimental approach cannot yet be justified in today's state of ignorance. Of equal importance, we must all beware that expensive, cumbersome, time-consuming, and even dangerous combinations of sophisticated hardware and antimicrobials not be allowed to supplant basic, sound patient management including an everyday intensive approach to infection prevention and management which is possible in any hospital setting.

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# Tissue Disposition of $^3$H-Actinomycin D (NSC-3053) in the Rat, Monkey, and Dog[1,2,3,4]

**Wayne M. Galbraith[5] and L. Bruce Mellett[6,7]**


## SUMMARY

**Tissue concentrations of $^3$H-actinomycin D have been determined in the rat, monkey, and dog after an iv dose of 0.6 mg/m$^2$ body surface area. The drug-tissue half-life was determined for various tissues of the rat, monkey, and dog. A mean drug-tissue half-life of 47 hours was calculated for the tissues of the dog. Exceptions were the testis and brain. Significant concentrations of $^3$H-actinomycin D failed to accumulate in the brain. Although testis drug concentrations were lower than most other tissues evaluated, the drug-tissue half-life was significantly greater than that of other tissues. In all species studied $^3$H-actinomycin D was rapidly depleted from serum after iv dosage, with concomitant accumulation of drug in the tissues. $^3$H-actinomycin D was excreted via the biliary and urinary routes in all species studied. No metabolites of $^3$H-actinomycin D were detected in the bile or urine of the rat, monkey, or dog with the methods employed. When expressed on a body weight basis, body surface area doses were more than threefold greater in the rat than in the dog. However, the average ratio of concentration × time values (rat C × T/dog C × T) for 11 different tissues of the rat and dog was only 1.3. The results strongly suggest that an equivalent dose of actinomycin D, with dosage based on a body surface area basis, results in nearly equal tissue-drug exposure for most tissues in various mammalian species.**


[Cancer Chemother Rep 59:1061-1069, 1975]

Earlier reports (1-7) of the tissue disposition of actinomycin D indicated that after iv dosage, this drug is rapidly removed from the blood due to tissue uptake and excretion. Urinary and biliary excretion of the drug has been reported (3,7), and no metabolism of actinomycin D has been observed in previous studies (3,6,7). All previous studies have also reported insignificant concentrations of actinomycin D in the brain.

The intracellular distribution has been studied by a number of investigators. Results of these investigations indicate that actinomycin D is localized in the cell nucleus. Actinomycin D does not appear to be bound appreciably to cellular constituents other than DNA (3,8,9). A study (10) of the binding of actinomycin D to rat serum proteins indicates that only about 8% of the drug is bound to serum proteins.

Dose-mortality studies and maximum tolerated doses of actinomycin D have been reported (11,12). Evaluation of these data on a dose/m$^2$ of body surface area (BSA) basis shows a noticeable relationship (12). The LD10s of actinomycin D for several mammalian species are remarkably similar to the total maximum tolerated dose in man. The data suggest that an equivalent dose of actinomycin D on a BSA basis in different mammalian species results in an equivalent toxicologic response (13,14).

As an interspecies relationship of dosage with respect to toxicity has been indicated (12,13) when dosage is based on an mg/m$^2$ of BSA basis, it was

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[3]Portions of this work were reported in abstract form (Pharmacologist, vols 15 and 16, 1973 and 1974) and in the doctoral dissertation of Dr. Galbraith (Department of Pharmacology, Graduate School, University of Alabama, Birmingham).

[4]Actinomycin D was supplied by the DCT, NCI; $^3$H-labeled actinomycin D (6.5 curies/mmol) was purchased from Schwarz/Mann, Inc, Orangeburg, NY.

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decided to compare tissue-drug distribution in each species after the administration of equivalent dosage on a BSA basis.

## MATERIALS AND METHODS

An appropriate volume of $^3$H-actinomycin D solution was evaporated to dryness to remove any volatile radioactivity. The residue was taken up into absolute ethanol (USP, reagent quality). The ethanol was then added to an appropriate amount of nontritiated actinomycin D. After completion of the dissolution of nontritiated actinomycin D in the ethanol, sterile saline (900 mg sodium chloride/100 ml) was added so that the final solution was one part ethanol and four parts saline. The injection solution was prepared in such a manner that not more than 1 ml of the injection solution contained the amount of actinomycin D to be injected/kg of body weight (BW). At all times, exposure of solutions of the drug to light was minimized since there is some degradation of actinomycin D when it is exposed to direct illumination.

### Experimental Animals

The animals utilized in these studies were $BDF_1$ mice, Sprague-Dawley rats, beagle dogs, and rhesus monkeys *(Macaca mulatta)*. All animals were fasted 12 hours before drug administration, but water was permitted ad libitum.

BSA for all animals in this study was determined as described by Freireich et al (12). All rats for which tissue levels were reported weighed between 100 and 150 g. Larger rats were used to evaluate the excretion of $^3$H-actinomycin D in the bile and saliva. The average dog weight was 11.3 kg; the average monkey weight was 3.6 kg.

The drug was administered via the femoral vein to rats anesthetized with methoxyflurane. After drug administration the skin incision exposing the vein was closed and the animal was allowed to regain consciousness. The drug was administered to conscious dogs and monkeys via the cephalic vein.

### Sample Collection

*Rat.*—Rats in which urine levels were evaluated were restrained before drug administration and a urinary catheter was placed in the urethra and secured. At a selected time after drug administration, the rats were killed and tissue samples collected. Serial blood samples from the same animals were collected via cardiac puncture. Saliva was collected from the rat between 3 and 6 hours after drug administration. The procedures used for the collection of saliva from the rat have been described by Yoshida et al (15) and Schneyer and Yoshida (16). For biliary excretion studies male Sprague-Dawley rats were anesthetized with methoxyflurane and a laparotomy was performed. The bile cannula was secured in the bile duct with cyanoacrylate resin (Locktite quick-set adhesive 404). The surgical wound was then sutured. A heating lamp was used to aid the animal in the maintenance of a normal body temperature.

*Dog and monkey.*—Urine was collected via a Foley catheter. The bladder was irrigated with 5 ml of sterile water after each collection period. The wash was then added to collected urine.

Exsanguination was the method employed to kill the rats, monkeys, and dogs. All animals were anesthetized with methoxyflurane or pentobarbital before being killed. Mice were killed by cervical dislocation.

### Chromatography of $^3$H-Actinomycin D

The paper and thin-layer chromatography systems in which injection solutions, urine, and bile samples were chromatographed have been reported (17,18) previously.

### Biliary Excretion Study (Dog)

The common bile duct of the beagle dog was joined by two cannulae, one draining from the liver and the other directed toward the intestine. The cystic duct was tied off and the gall bladder of the dog was removed. The two cannulae were connected on the exterior of the animal so that bile would flow into the intestine when not being collected. When the biliary samples were collected, an equal volume of a physiologic solution (lactated Ringer's, USP) was injected into the intestine to replace lost fluid and electrolytes. The surgical wound was allowed to heal for 5 days before drug administration. After drug dosage, biliary and urinary samples were collected from conscious dogs which were immobilized by utilization of a specially designed sling.

### Homogenization of Whole Mouse Carcass

Nine ml of water:ethyl alcohol (4:1 ratio)/g of BW was added to the mouse carcass in a beaker. A Brinkman Polytron homogenizer was used to homogenize the whole animal.

### Tissue Lyophilization

Nine ml of water/g of tissue weight was added to the selected tissue in a beaker. A Brinkman Polytron homogenizer was used to homogenize the tissue. The total radioactivity of homogenate was determined

and the sample was then lyophilized. After lyophilization the sample was reconstituted with water and the total radioactivity of the reconstituted homogenate was determined.

### Total Radioactivity

Total radioactivity of tissues, serum, urine, and bile was determined after first adding 2 ml of tissue solubilizer (Soluene-350, Packard) to a 100-mg sample in a scintillation counting vial. Other aspects of the procedure are described in the Packard brochure on Soluene-350.

Radioactivity was evaluated in a Packard Tri-Carb liquid scintillation spectrometer. An external standard was employed to correct for quenching. Counts for all samples were greater than twice background. With the exception of serial serum samples from the rat, all samples were counted in duplicate and the average value was used to determine tissue drug concentration. With one exception,[8] samples with counting efficiencies of <20% were not used.

### Dose Expression

This paper refers to dosage based on both mg/m$^2$ BSA and mg/kg BW. In table 1, dosage is expressed using both bases for five mammalian species at the dose level we employed in this study.

## RESULTS

### Body Balance of Radioactivity ($BDF_1$ Mouse)

At selected times (injection time to 48 hours after injection) after the ip administration of 0.6 mg/m$^2$ BSA $^3$H-actinomycin D, mice were killed and homogenized. Excreta from the mice were collected in metabolism cages for the various time periods. Mice killed 24 and 36 hours after drug administration had excreted 35% and 54% of the administered drug by the time of death. The biologic half-life of $^3$H-actinomycin D in the $BDF_1$ mouse was estimated to be 33 hours. When total radioactivity in each mouse carcass was determined and added to radioactivity excreted in the urine and feces for various time periods, > 90% of the administered radioactivity was accounted for in all animals studied.

### Tissue Lyophilization

A comparison of the total radioactivity of the tissue homogenates of dog tissues with the total radioactivity of the same homogenates after lyophilization and reconstitution showed no significant differences in radioactivity.

### Metabolism

Bile and urine samples from rats, monkeys, and dogs that had been given $^3$H-actinomycin D were chromatographed using the thin-layer and paper chromatography systems previously referenced in the Methods section. Similar chromatograms were obtained from the injection solutions. No evidence of metabolism of $^3$H-actinomycin D by the rat, monkey, or dog was observed. These results were consistent with previous reports from studies in the rodent (3,6,7).

The similarity of drug disposition kinetics in the rat, monkey, and dog adds evidence that the metabolic fate of actinomycin D is similar in all three species.

### Tissue Disposition

Serum levels of $^3$H-actinomycin D in rats, monkeys, and dogs were rapidly depleted after iv administration with a concomitant accumulation of drug

[8]Some spleen samples had efficiencies of slightly <20%; the counting efficiencies of the other tissues were 35%-4. %.

TABLE 1.—Comparison of dosage calculated on the basis of BSA and BW*

| Species | BW (kg) | BSA (m$^2$) | Dosage based on— mg/m$^2$ | mg/kg |
|---|---|---|---|---|
| Mouse | 0.020 | 0.0066 | 0.6 | 0.200 |
| Rat | 0.150 | 0.025 | 0.6 | 0.102 |
| Monkey | 3.0 | 0.24 | 0.6 | 0.050 |
| Dog | 8.0 | 0.40 | 0.6 | 0.030 |
| Man, child | 20.0 | 0.80 | 0.6 | 0.024 |
| Man, adult | 60.0 | 1.6 | 0.6 | 0.016 |

*Adapted from ref 12.

in the tissues. Serum levels were compared after a dose of 0.6 mg/m² BSA in the rat, monkey, and dog (fig 1). The serum concentrations for the rat in figure 1 represent serial serum samples from the same rat.

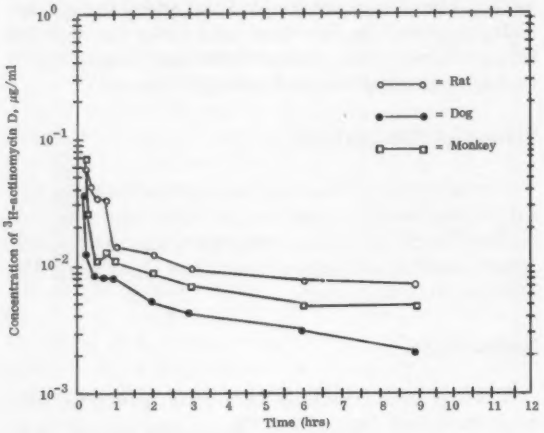


FIGURE 1.—Serum levels of drug in animals treated with 0.6 mg/m² iv of ³H-actinomycin D.

The drug distribution phase was estimated to be approximately 3 hours in all species. This time was chosen because the second phase of a clearly biphasic serum concentration curve appears to be initiated about 3 hours after dosage in all species.

After the distribution phase,[9] tissue-drug concentrations (with the exception of the testis and brain) were maintained at values at least ten times that of serum.

During the first 9 hours after drug dosage serum levels of the smaller animal species were higher than in the larger animal species after an equivalent dose on the basis of BSA. The higher serum-drug concentrations in small animals were not surprising since these animals received a higher dose on a BW basis than the larger animals, and one would therefore expect a higher initial concentration of the drug.

³H-actinomycin D was found to be excreted via the biliary and urinary routes in the rat, monkey, and dog. The biliary excretion was not surprising in view of the high lipid solubility and high molecular weight of the drug.

In table 2, the excretion of actinomycin D by the rat, monkey, and dog was compared. The data indicated that a greater percentage of dose was excreted from the smaller animals than from the larger animals during the first 24 hours after dosage.

Saliva was collected from the submaxillary salivary gland between 3 and 6 hours after an iv dose of 100 μg/kg of ³H-actinomycin D in the rat. No radioactivity was detected in the saliva despite the high concentrations of drug in the submaxillary gland during the time period studied.

Tissue levels of ³H-actinomycin D for selected tissues in the rat, monkey, and dog after an iv dose of 0.6 mg/m² BSA were graphically compared in figures 2-4.[10] A similar pattern was observed for most other tissues.[11]

[9]Distribution phase is defined as the initial period of tissue-drug uptake after drug administration.

[10]Drug-tissue levels in the rat between 9 and 144 hours represent average values for three rats. Standard errors (SE) for all average values were $< \pm 0.010$ with the exception of spleen at 9 and 24 hours ($\leq \pm 0.033$), and small intestine at 9 hours, lung at 24 hours, stomach at 24 hours, and thymus at 72 and 144 hours, which were $\leq \pm 0.017$. At 48 and 72 hours all SE were $\leq \pm 0.006$ with the exception of spleen at 74 hours ($\pm 0.009$). SE for all values at 96, 120, and 144 hours were $\leq \pm 0.002$ with the exception of spleen ($\leq \pm 0.006$), thymus ($\leq \pm 0.008$), and small intestine at 96 hours ($\pm 0.009$). All values for the dog and monkey represent one animal at the time indicated.

[11]Data for other tissues will be supplied upon request.

TABLE 2.—Excretion of ³H-actinomycin D by various species

| Excretion from— | Time after drug administration (hrs) | Drug excreted (%) in— | | |
|---|---|---|---|---|
| | | Rat | Monkey | Dog |
| Urine | 0-6 | 15.0 | 12.9 | 6.4 |
| | 0-12 | 19.6 | 16.9 | 8.4 |
| | 0-24 | 31.0 | 22.1 | 11.9 |
| Bile | 0-6 | 11.6 | 8.9* | 5.0 |

*Bile was collected from a monkey anesthetized with sodium thiamylal; in other respects the collection of bile from the monkey was performed in a manner similar to that described for the rat and dog.

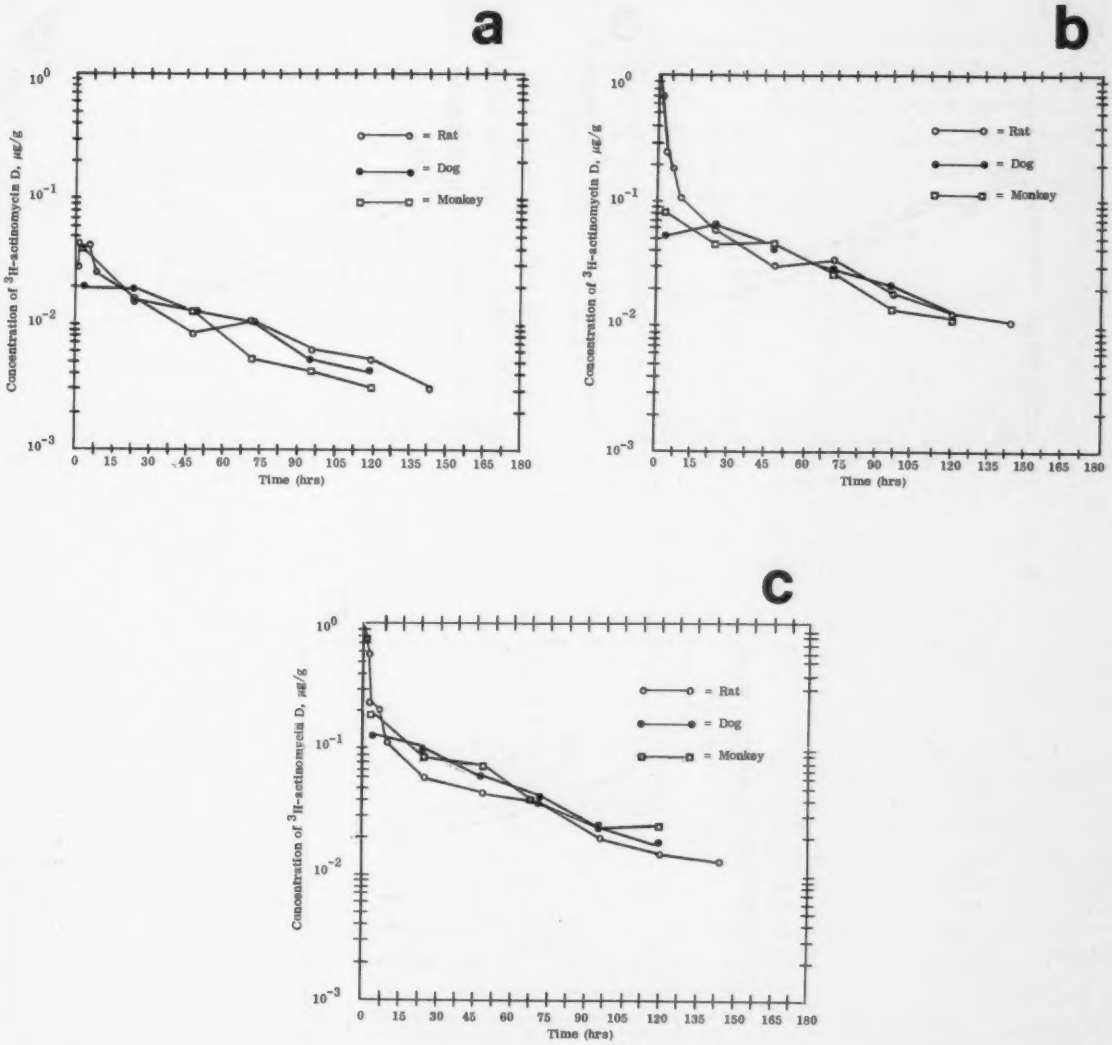


FIGURE 2.—Tissue levels of drug in animals treated with 0.6 mg/m² iv of $^3$H-actinomycin D. *a* = Skeletal muscle; *b* = lung; *c* = kidney.

Drug disposition in the testis was markedly different from other tissues in all species. Initial drug concentrations in the testis were lower than those for most other tissues. However, the rate of drug elimination from this tissue was much slower than for other tissues. This same phenomenon was noted for thymus drug concentrations in the rat.

The concentration of $^3$H-actinomycin D found in the dog, monkey, and rat brain was consistent with serum levels at the time of evaluation.

The linearity of joined data points on semilogarithmic coordinates clearly indicated first-order elimination of actinomycin D from the tissues; ie, a constant fraction of the drug in the tissues was eliminated at each time interval.

Regression lines were calculated for the elimination phase of all tissues in the rat, monkey, and dog. The regression line was used to calculate the tissue-drug half-life of $^3$H-actinomycin D. The results are given in table 3.

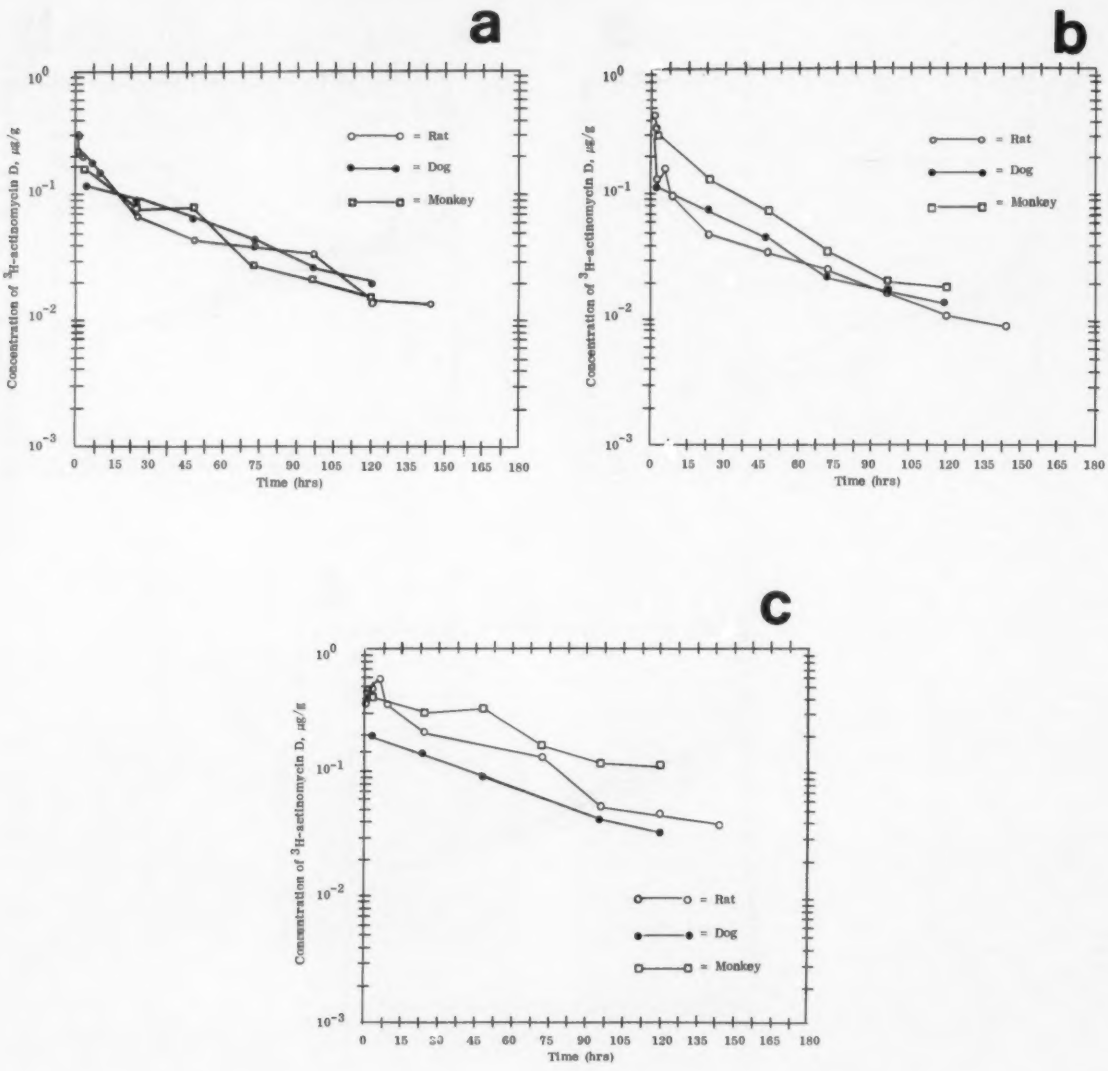


FIGURE 3.—Tissue levels of drug in animals treated with 0.6 mg/m² iv of $^3$H-actinomycin D. *a* = Small intestine; *b* = liver; *c* = spleen.

The error in determining the regression line for bone marrow was significantly greater than that for other tissues. This is probably related to the difficulty in sampling and the lack of uniformity between samples.

A test for equality of slopes (95% probability) (19) was used to compare the rate of elimination of $^3$H-actinomycin D from individual tissues in the different species. The results suggested that the same slope, and therefore the same half-life, could be used to describe drug elimination of the same tissue in the rat, monkey, and dog.

A test for equality of slopes failed to demonstrate a significant difference in the slopes of the regression lines describing the drug elimination of different tissues of the same species. This suggested that the same half-life may be useful to describe the elimination of $^3$H-actinomycin D from different tissues of the rat, monkey, and dog.

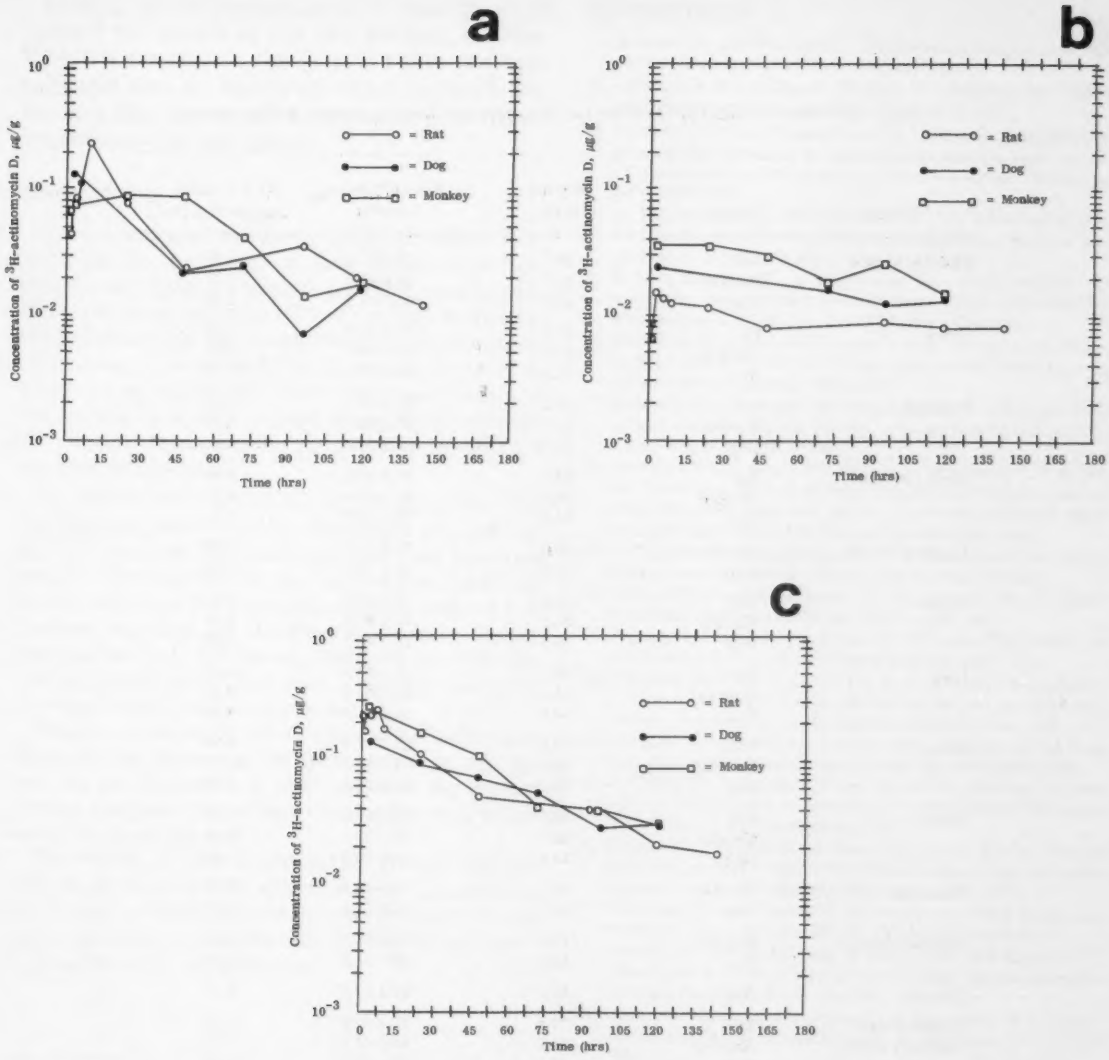


FIGURE 4.—Tissue levels of drug in animals treated with 0.6 mg/m² iv of $^3$H-actinomycin D. *a* = Bone marrow; *b* = testis; *c* = submaxillary salivary gland.

The drug-tissue half-life calculated from the mean slope of the tissues of the dog was 47 hours. In view of its high lipid solubility, lack of biotransformation, and strong binding to DNA, the long tissue half-life of $^3$H-actinomycin D is not surprising.

## Tissue-Drug Exposure

Regression lines describing the elimination of $^3$H-actinomycin D from the tissues of the rat, monkey, and dog were transposed from semilog plots to arithmetic plots. On both plots the y axis represented μg of drug/g of tissue and the x axis represented time in hours. The area under the regression line was calculated between zero time and time at which the drug concentration in the tissue reached 0.005 μg/g. This value was chosen because an in vitro actinomycin D concentration of 0.005 μg/ml had been reported to inhibit synthesis of ribosomal RNA by mammalian cells (20).

TABLE 3.—Half-life and tissue C × T values for $^{3}$H-actinomycin D in rat, monkey, and dog tissues*

| Tissue | Species | Half-life (hrs) | 95% Confidence interval | C × T under regression line† |
|---|---|---|---|---|
| Skeletal muscle | Rat | 38.7 | 29.1-57.7 | 1.60 |
| | Monkey | 32.7 | 24.5-49.3 | 1.23 |
| | Dog | 49.3 | 36.9-74.5 | 1.24 |
| Heart | Rat | 30.7 | 22.6-47.8 | 4.48 |
| | Monkey | 31.7 | 20.6-69.2 | 2.43 |
| | Dog | 38.2 | 31.2-49.4 | 2.17 |
| Stomach | Rat | 35.2 | 28.0-47.3 | 4.73 |
| | Monkey | 44.5 | 35.5-59.5 | 4.19 |
| | Dog | 51.7 | 45.2-60.7 | 5.13 |
| Small intestine | Rat | 33.9 | 27.0-45.6 | 8.59 |
| | Monkey | 34.7 | 26.1-51.8 | 7.26 |
| | Dog | 44.5 | 39.4-50.9 | 8.31 |
| Large intestine | Monkey | 38.6 | 27.9-62.5 | 5.85 |
| | Dog | 41.7 | 36.0-49.6 | 5.41 |
| Kidney | Rat | 27.6 | 19.9-45.2 | 8.75 |
| | Monkey | 38.1 | 27.2-63.4 | 8.91 |
| | Dog | 38.8 | 35.2-43.1 | 7.98 |
| Lung | Rat | 26.1 | 17.8-48.5 | 14.47 |
| | Monkey | 41.4 | 31.4-60.6 | 8.44 |
| | Dog | 54.6 | 37.3->100 | 9.34 |
| Liver | Rat | 29.0 | 21.7-43.9 | 6.29 |
| | Monkey | 28.3 | 22.0-39.7 | 10.57 |
| | Dog | 36.2 | 29.3-47.3 | 5.87 |
| Spleen | Rat | 36.5 | 30.6-45.2 | 24.06 |
| | Monkey | 56.0 | 39.1-99.1 | 35.88 |
| | Dog | 44.6 | 39.9-50.6 | 12.60 |
| Pancreas | Monkey | 37.1 | 30.0-48.6 | 13.41 |
| | Dog | 39.1 | 30.5-54.6 | 9.23 |
| Adrenal gland | Monkey | 36.0 | 26.7-55.7 | 4.30 |
| | Dog | 45.3 | 38.7-54.6 | 3.13 |
| Thyroid | Dog | 50.3 | 49.4-51.2 | 9.12 |
| Submaxillary salivary gland | Rat | 37.2 | 30.4-47.9 | 11.24 |
| | Monkey | 34.7 | 26.2-51.0 | 9.18 |
| | Dog | 46.9 | 36.5-65.6 | 12.68 |
| Bone marrow | Rat | 47.0 | 30.6-> 100 | 6.80 |
| | Monkey | 42.9 | 24.6-> 100 | 6.63 |
| | Dog | 34.9 | 15.9-> 100 | 4.48 |

*Dose = 0.6 mg/m$^{2}$ BSA iv; C × T = $\mu$g/g × hrs.

†C × T values represent the area under the regression line from 0 time to the time at which a minimum effective tissue-drug concentration (0.005 $\mu$g/g) was attained; regression line was drawn using only data points after 3 hrs.

Table 3 gives concentration × time (C × T) values[12] for tissues of the rat, monkey, and dog. With few exceptions, the C × T values strongly suggested that an equivalent dosage in each species on a BSA basis resulted in an equivalent tissue-drug exposure in each species.

## DISCUSSION

There is impressive evidence (12,21-23) which demonstrates the usefulness of drug dosage based on BSA for interspecies comparisons of pharmacologic effect and drug exposure (C × T) in serum. The results of this study add to this evidence. In this study tissue-drug exposure in different species is related to dosage on an mg/m² BSA basis. Previous studies of this nature have only related serum drug exposure (C × T) with dosage on an mg/m² basis in different mammalian species.

The difference in BW of rats and dogs used in this investigation was > 75-fold. Expressed on a BW basis, the 0.6-mg/m² BSA dose was more than threefold greater in the rat than in the dog. Despite the difference in dose on a BW basis, the average ratio of C × T values (rat/dog) for 11 different tissues of the rat and dog was only 1.3 (range, 2.06-0.89; SD, 0.39) following the 0.6-mg/m² BSA dose. The ratio was > 1.5 for only four of the tissues evaluated.

We have also employed a higher dose in the dog. When similar doses (on an mg/kg basis) in the rat and dog are compared, C × T values of dog tissues are approximately five times the C × T values of the same tissues of the rat.

The results of this investigation clearly suggest that an equivalent dose of actinomycin D, with dosage based on BSA, results in nearly equal tissue-drug exposure in mammalian species which have large differences on body size.

[12]This formula was used to calculate C × T values:

$$C \times T = \frac{\text{Half-life}}{0.693} \times [C_o - C_{min}]$$

$C_o$ = Concentration extrapolated to zero time
$C_{min}$ = Minimum cytotoxic concentration

After transposing regression lines from semilog plots to arithmetic plots, chart paper grids under the regression lines were counted in order to determine C × T values and verify the accuracy of the formula.

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# Toxicology Studies With Cytembena (NSC-104801), an Antineoplastic Agent With a Multispecies Nephrotoxic Effect[1,2,3]

Edward J. Gralla,[4] Gerald L. Coleman, George W. Osbaldiston, Michael Kashgarian, and Albert M. Jonas[5,6]

## SUMMARY

**The toxic effects of cytembena in beagle dogs and rhesus monkeys were investigated with the drug given as single or daily iv injections in doses ranging from 12.5 to 200 mg/kg/day to dogs and 6.25 to 50 mg/kg/day to monkeys. Renal tubular damage was a major drug- and dose-related finding in both species and was clinically indicated by an accompanying uremia, elevated serum creatinine, and proteinuria. In the kidney, the primary lesion was cellular necrosis and desquamation of the distal tubular epithelium in animals given the lowest toxic doses. More severe but similar histologic changes which involved both the proximal and distal tubules were found following lethal or sublethal doses in both species. The early subcellular renal changes produced by this drug were further characterized by single dose studies in mice which showed renal mitochondrial swelling and disruption plus generalized cell swelling as progressive, subcellular developments which were well established 24 hours after treatment. Cellular regeneration in the renal tubular epithelium was found in dogs and monkeys retained 6 weeks for observation after treatment, although functional recovery was inconsistent. A toxic effect to lymphoid tissues was an additional finding which is described.**

[Cancer Chemother Rep 59:1071-1081, 1975]

Cytembena, the sodium salt of β-4-methoxybenzoyl-β-bromoacrylic acid (fig 1), emerged as the most promising candidate from a series of β-crotonolactone derivatives (1) which were synthesized and studied for possible anticancer activity. Among the reported antineoplastic properties of this compound are inhibition of tumor development and lifespan prolongation of mice carrying any one of the following experimentally induced tumors: Sarcoma 180, mammary adenocarcinoma, Ehrlich ascites carcinoma, Gardner lymphosarcoma, and methylacridine-induced sarcoma. Similar effects have been found in rats with transplanted Yoshida ascites sarcoma or Zajedela ascites hepatoma (2). Favorable clinical responses were reported in patients afflicted with a variety of solid tumors which included cervical (3), uterine, ovarian (4), and skeletal carcinomas and soft tissue sarcomas (5). Furthermore, since this agent produced no hematopoietic toxicity in any of these studies, it appeared to be a suitable agent for use in combination with more classic anticancer drugs (6,7). Single-dose toxicity studies in mice and rats and multiple-dose toxicity studies in rats with cytembena given parenterally were conducted and reported by Jelínek et al (2). The most pronounced toxic effects reported by these authors were renal changes described simply as "hypertrophy renal channels," which correlated with an increased kidney weight found in male and female rats given a dose of 50 mg/kg/day sc for 6 months. More evidence of a nephrotoxic potential was discovered in the clin-

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[2]Supported by contract NIH-70-2089 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Cytembena: CAS reg. No. 16170-75-5; acrylic acid, 3-p-anisoyl-3-bromo-, sodium salt; sodium-*cis*-β-4-methoxybenzoyl-β-bromoacrylate. Supplied by Drug Research and Development Program, DCT, NCI.

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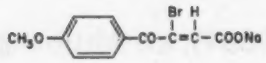

FIGURE 1.—Cytembena structure.

ical studies conducted by Skoda et al (8) who reported albuminuria and Nagel et al (9) who described proteinuria and uremia. Both groups of investigators independently reported that signs of a possible intestinal toxicity, nausea and vomiting, appeared in their patients.

An agent with antineoplastic properties against solid tumors would fill a critical need in cancer chemotherapy. At the same time there are serious risks in the widespread use of a drug with a poorly understood toxicity. The purpose of these investigations was to define experimentally the potential hazards associated with the use of cytembena. This report will call attention to and describe a multispecies renal toxic effect with histologic lesions located primarily in the distal tubules.

## METHODS

Lyophilized cytembena was supplied in sealed ampules of 200 mg/vial. Fresh solutions were prepared daily by dissolving the compound in sterile, distilled water to a concentration of 50 mg/ml (pH 7.4). The clear solution was then administered iv without further handling. The protocol for these studies followed the principles outlined by Prieur et al (10).[7]

Registered beagles, 5½-12 months of age, and conditioned *Macaca mulatta* monkeys weighing 3-5 kg, obtained from commercial sources, were housed in stainless-steel cages within temperature-humidity controlled rooms. Food and water were available ad libitum but were withheld 12 hours before blood and urine samples were taken. Immediately upon arrival, all animals were weighed and checked for physical defects. Monkeys were conditioned during a 4-week observation period when a hematologic analysis, a chest radiograph, and three biweekly tuberculin tests were performed.

Blood and urine were obtained from all animals for baseline determinations twice before dosing was initiated, at 2-day intervals during treatment, and weekly during post-treatment recovery. Analyses included hematocrit; hemoglobin; red blood cell, white blood cell, differential, platelet, and reticulocyte counts; alkaline phosphatase; serum glutamic oxaloacetic transaminase; serum glutamic pyruvic transaminase; total and direct bilirubin; sulfobromophthalein excretion; prothrombin time; blood urea nitrogen (BUN); creatinine; glucose; sodium; potassium; magnesium; calcium; and complete urinalysis. Physical examinations including body weight, body temperature, and ophthalmoscopic examinations were performed on all animals weekly, and at the same time lead I, II, and III electrocardiograms were taken with the animals sitting upright in a special restraining device (11).

The dogs and monkeys were paired by sex and treated according to the following dosage regimen. A single dose of cytembena was administered to each partner of a pair starting at a low dose of 25 mg/kg and increasing by twofold multiples to a maximum of 200 mg/kg. Similarly, separate pairs of dogs and monkeys were treated daily for 5 successive days at levels ranging from 12.5 to 100 mg/kg/day in dogs and from 6.25 to 50 mg/kg/day in monkeys. Two extended treatment studies were also carried out in pairs of dogs. In the first study single weekly doses of 50 or 100 mg/kg were given for 6 weeks. In a second study, pairs of beagles were treated with cytembena for five daily doses of 12.5, 25, or 50 mg/kg/day on alternate weeks for three weekly treatments with intervening weeks without treatment to permit recovery from any toxic effects.

At the end of each treatment regimen (table 1), one of the pair of animals was selected, anesthetized with pentobarbital sodium, killed by exsanguination, and necropsied. Organs were examined grossly and tissues (fig 2) were collected for microscopic examination. The second animal was retained for 45 days without further treatment and observed for delayed toxicity and/or regression of any toxicity which developed during treatment.

### Mice

Studies in separate groups of male Swiss-Webster mice (25-30 g) examined the following: (*a*) crystal formation as a possible toxicity mechanism; and (*b*) lesion development at the subcellular level. One group of ten mice was given 1% ammonium chloride solution to maintain their urine pH between 5.5 and 6.0. They were then injected iv with cytembena (100 mg/kg) and two mice per group were necropsied at 30 and 60 minutes, and 4, 8, and 24 hours later. Each animal was killed by an overdose of ether anesthesia; the abdomen was opened and both kidneys were quickly removed, cryostat-frozen, and sectioned. Ten micron longitudinal sections were made in the midrenal area and examined microscopically under polarized light and by phase contrast microscopy

[7]Conversions to drug concentration per body surface ($m^2$) which apear in this report were calculated by using the formula of Freireich et al (body weight [kg] × km factor = $m^2$) (ref 12).

TABLE 1.—Summary of results: major toxic effects*

| Species | Regimen | No. of animals | Dose levels Mg/kg/day | Dose levels Mg/m²/day | Mortality | Nephrotoxicity | Lymphoid depletion | Weight loss | Emesis |
|---|---|---|---|---|---|---|---|---|---|
| Dog | Single dose | 2 | 200 | 4000 | 2 | 2 | 1 | 2 | 2 |
| | | 2 | 100 | 2000 | 0 | 2 | 0 | 2 | 2 |
| | | 2 | 50 | 1000 | 0 | 1 | 0 | 0 | 0 |
| | | 2 | 25 | 500 | 0 | 0 | 0 | 0 | 0 |
| | 5 daily doses | 2 | 100 | 2000 | 2 | 2 | 2 | 2 | 2 |
| | | 2 | 50 | 1000 | 0 | 2 | 1 | 2 | 2 |
| | | 2 | 25 | 500 | 0 | 1 | 0 | 1 | 0 |
| | | 2 | 12.5 | 250 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Once wk × 6 wks | 2 | 100 | 2000 | 1 | 2 | 1 | 2 | 2 |
| | | 2 | 50 | 1000 | 0 | 2 | 0 | 1 | 1 |
| | 5 daily doses, 9-day rest × 3 | 3† | 50 | 1000 | 1 | 2 | 1 | 2 | 2 |
| | | 2 | 25 | 500 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | 12.5 | 250 | 0 | 0 | 0 | 0 | 0 |
| Monkey | 5 daily doses | 2 | 50 | 600 | 1 | 2 | 1 | 0 | 2 |
| | | 2 | 25 | 300 | 0 | 2 | 1 | 0 | 1 |
| | | 2 | 12.5 | 150 | 0 | 1 | 0 | 0 | 0 |
| | | 2 | 6.25 | 75 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Two animals, 1 male and 1 female, were included in each group. The numbers indicate the number of animals responding in each group.
†Three animals treated; 1 died after the first sequence.

| | | | | | | |
|---|---|---|---|---|---|---|
| Adrenal | Colon | Gallbladder | Injection site | Lymph node | Rib | Stomach |
| Brain | Diaphragm | Gonads and | Jejunum | Optic nerve | Salivary gland | Striated muscle |
| Breast | Duodenum | accessories | Kidney | Pancreas | Spinal cord | Thymus |
| Bone Marrow | Esophagus | Heart | Liver | Parathyroid | Skin | Thyroid |
| Cecum | Eye | Ileum | Lungs | Pituitary | Spleen | Urinary bladder |

FIGURE 2.—Tissues examined.

for evidence of foreign crystals. Control mice that received tap water were processed similarly. Ultrastructural studies were performed in a third group of four mice maintained on tap water injected in the same manner. Eight and 24 hours later they were killed by an overdose of ether and the kidneys were removed, immediately fixed in gluteraldehyde, and processed for electron microscopic examination of the tubular regions.

## RESULTS

### Nephrotoxicity

Cytembena proved to be lethal in dogs (table 1) given single doses of 200 mg/kg or five daily doses of 100 mg/kg. In monkeys, a dose of 50 mg/kg given daily for 5 days was fatal. In both species, renal failure was judged to be the ultimate cause of death. This conclusion was based on clinical and pathologic signs which developed in both species, such as uremia, elevated serum creatinine, hematuria, and proteinuria, plus dose-related toxic lesions found in the renal tubules at necropsy (table 2).

Grossly the kidneys were of normal size and color. When sliced transversely the cortices appeared pale and the cut surfaces bulged slightly above the capsule. Microscopically throughout the kidneys of both dogs and monkeys receiving the highest toxic doses, few of the distal convoluted tubular epithelial cells remained and these were swollen with pyknotic nuclei and basophilic cytoplasm. The tubular lumina contained granular or amorphous eosinophilic cellular debris. In some nephrons a similar form of necrosis extended into the proximal tubules as well. Collecting tubules were undamaged in all treated animals. At the lower doses necrosis was progressively less evident and involved only the distal tubules. Minimally affected tubules were lined by enlarged cells containing basophilic cytoplasm, hydropic swelling, and enlarged nuclei (fig 3). In addition, focal scattered areas had accumulations of small mononuclear cells located adjacent to tubules with wrinkled tubular basement membranes. PAS-positive amorphous or granular casts were found within the lumens of denuded tubules. Elsewhere, scattered enlarged tubular cells containing basophilic cytoplasm or mitotic figures indicated regeneration had been taking place were present in animals killed more than 5 days after treatment.

These renal changes followed a dose response pattern (fig 4, table 1), with the most extensive damage

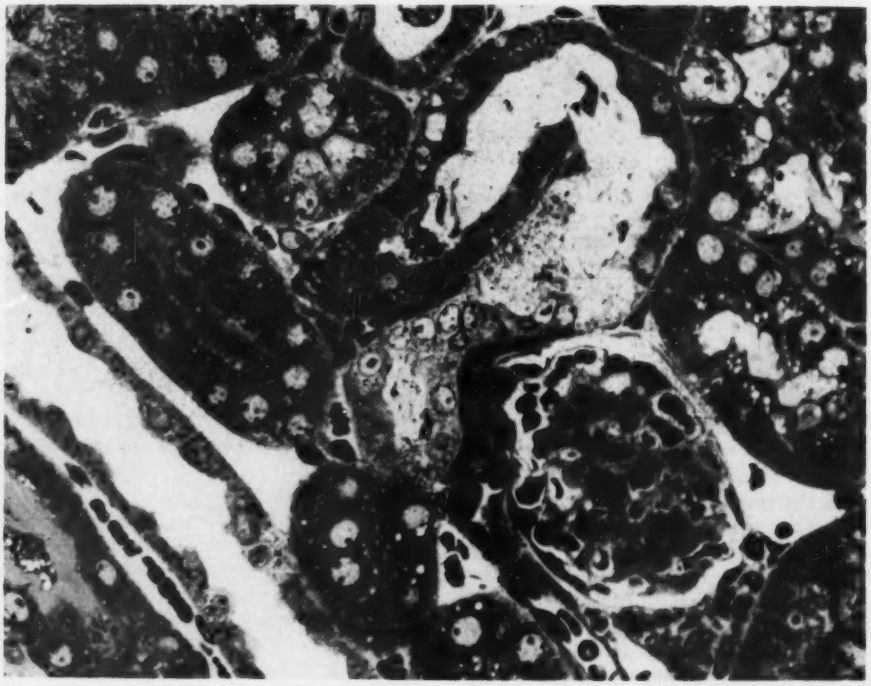

FIGURE 3.—One-micron section of renal cortex showing focal hydropic swelling of the epithelial cells lining distal tubule (toluidine blue stain, × 250).

TABLE 2.—Nephrotoxic signs in dogs and monkeys given toxic doses of cytembena

| Animal No./ sex | Species | Dose | | | BUN (mg/100 ml) on Day— | | | Creatinine (mg/ 100 ml) on Day— | | | Renal tubular lesions (location) | | Description |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Daily (mg/kg) | Total No. | Total Mg/kg | 0* | 6 | 30 | 0* | 6 | 30 | Distal | Proximal | |
| QITBW/F† | Dog | 100 | 1 | 100 | 16 | 82 | — | 0.21 | 0.95 | — | + | + | Denuded tubules with cellular debris, occasional casts |
| QOEAV/M‡ | Dog | 100 | 1 | 100 | 19 | 87 | 47 | 0.21 | 1.40 | 0.37 | + | + | Dilated tubules with flattened epithelium |
| 4915/F† | Dog | 50 | 1 | 50 | 8 | 13 | — | 0.20 | 0.19 | — | – | – | No drug-related abnormalities |
| 4918/M‡ | Dog | 50 | 1 | 50 | 14 | 15 | 17 | 0.18 | 0.17 | 0.22 | – | – | No drug-related abnormalities |
| ZBUCL/M† | Dog | 50 | 5 | 250 | 13 | 81 | — | 0.40 | 7.80 | — | + | + | Cell necrosis and desquamation |
| ZJCBM/F‡ | Dog | 50 | 5 | 250 | 22 | 21 | 8 | 0.32 | 0.90 | 0.97 | – | – | No abnormalities |
| MMEBX/F† | Dog | 25 | 5 | 125 | 19 | 26 | — | 0.56 | 1.10 | — | + | – | Swollen basophilic lining cells |
| ZHZBT/M‡ | Dog | 25 | 5 | 125 | 20 | 14 | 15 | 0.42 | 1.10 | 1.0 | – | – | No drug-related abnormalities |
| ZHZBT/3M† | Dog | 12.5 | 5 | 62.5 | 17 | 22 | — | 0.77 | 0.88 | — | – | – | No drug-related abnormalities |
| QORBS/F‡ | Dog | 12.5 | 5 | 62.5 | 19 | 19 | 14 | 0.65 | 0.85 | 0.85 | – | – | No drug-related abnormalities |
| 379/M† | Monkey | 50 | 5 | 250 | 31 | 83 | — | 0.34 | 4.2 | — | + | + | Cell necrosis and desquamation |
| 375/F§ | Monkey | 50 | 5 | 250 | 28 | 82 | — | 0.31 | 3.8 | — | + | + | Denuded tubules with cellular debris |
| 387/M† | Monkey | 25 | 5 | 125 | 23 | 50 | — | 0.25 | 2.2 | — | + | – | Tubules denuded or with flattened epithelium |
| 392/F‡ | Monkey | 25 | 5 | 125 | 20 | 85 | 22 | 0.25 | 3.0 | 0.25 | + | – | Shrunken tubules; enlarged lining cells |
| 360/F† | Monkey | 12.5 | 5 | 62.5 | 22 | 72 | — | 0.29 | 0.77 | — | + | – | Dilated tubules with flattened epithelium filled with necrotic cells |
| 393/M‡ | Monkey | 12.5 | 5 | 62.5 | 22 | 25 | 16 | 0.25 | 0.23 | 0.31 | – | – | No abnormalities |
| 378/F† | Monkey | 6.25 | 5 | 31.25 | 21 | 12 | — | 0.26 | 0.14 | — | – | – | No abnormalities |
| 382/M‡ | Monkey | 6.25 | 5 | 31.25 | 16 | 10 | 26 | 0.27 | 0.20 | 0.25 | – | – | No abnormalities |

*Average of 2 determinations taken 7-10 days apart.
†Killed and necropsied on Day 6.
‡Killed and necropsied 42 days after last treatment.
§Died on Day 6.

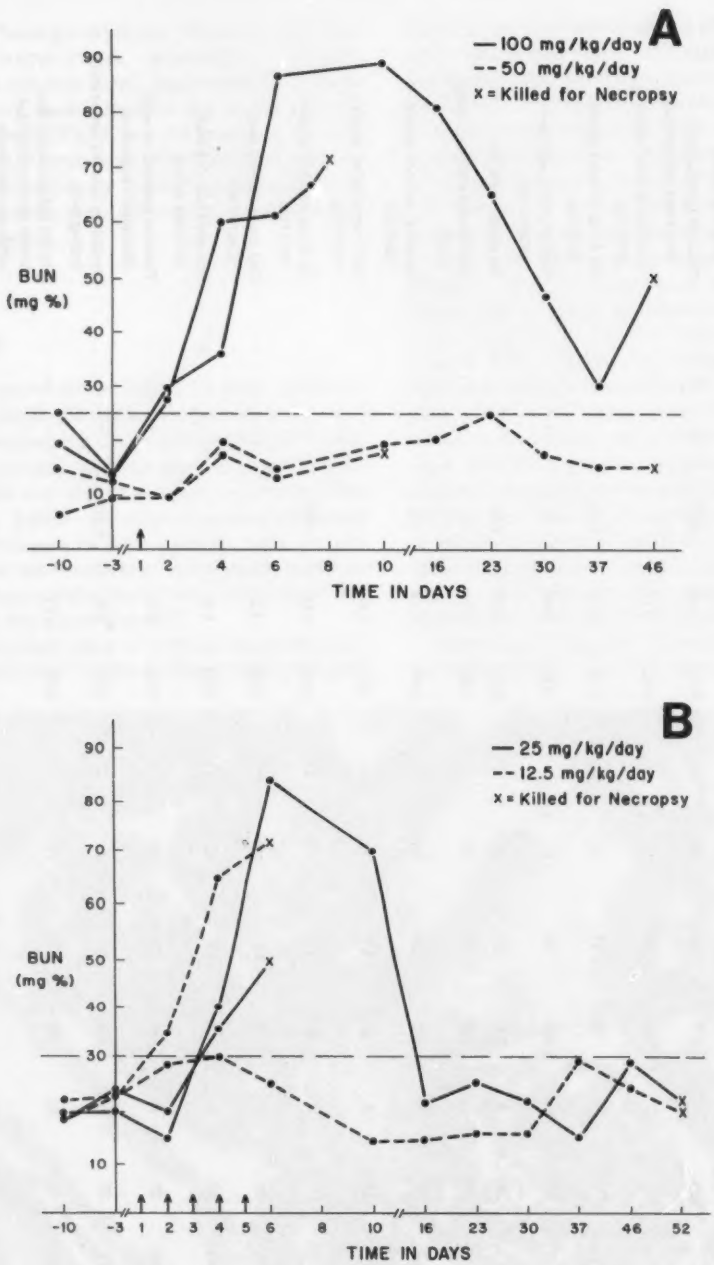


FIGURE 4.—A. BUN levels after a single injection of cytembena to beagle dogs. A dose of 100 mg/kg/day causes a functional impairment in 2 injected dogs which is incompletely restored following cessation of treatment. A dose of 50 mg/kg/day has no effect. The broken horizontal line is upper limit of normal; the arrow indicates drug injection. B. BUN levels after 5 daily iv injections of cytembena to rhesus monkeys. A dose of 25 mg/kg/day causes values to rise significantly above the upper limit of normal (broken horizontal line) in 2 injected monkeys. A dose of 12.5 mg/kg/day produces the same effect in 1 of 2 monkeys that were injected. Functional recovery occurs within 12 days. The arrows indicate drug injections.

occurring at the highest dose levels. Clinical signs of renal insufficiency, uremia, proteinuria, hematuria, and elevated creatinine were seen at the highest dose levels only. At the lowest toxic doses, renal cellular damage was observed microscopically which had not been predicted by laboratory analyses (table 2).

No evidence of drug crystallization was found in the renal tubules of mice treated with cytembena (100 mg/kg iv) which were excreting either normal (pH 6.5-8.0) or acidified (pH 5.5-6.0) urine. The kidneys from treated mice which were examined by electron microscopy 8 hours after a single iv injection of 100 mg/kg of cytembena showed early evidence of cell damage as indicated by decreased cytoplasmic density and occasional mitochondrial swelling. At this time toxic changes were limited to the distal tubule (fig 5). Twenty-four hours after receiving cytembena widespread signs of subcellular destruction were present which were characterized by apical swelling in cells showing decreased cytoplasmic density. Mitochondria were separated and distorted with many showing fragmented cristae and ruptured limiting membranes. These signs were present in both proximal and distal tubules (fig 6). In addition, an occasional cell contained flocculent electron densities within the nucleus (fig 6). A rare finding was swelling of the endoplasmic reticulum (fig 7).

### Lymphoid Toxicity

Damage to lymphoid tissues occurred only at the highest dose levels (table 1) in both dogs and monkeys. All sensitive tissues, including lymph nodes, tonsils, thymus, and spleen, were involved. Lesions were characterized by loss of cellularity and reduced numbers of mitotic figures in the germinal centers. Relative and absolute numbers of lymphocytes in the peripheral circulation were unaffected.

### Gastrointestinal Toxicity

Emesis occurred immediately after treatment in dogs and monkeys given the highest dose level (table 1). This was followed by anorexia and a gradual weight loss which was quickly regained after treatment was stopped. No microscopic indications of gastrointestinal cytotoxicity were observed.

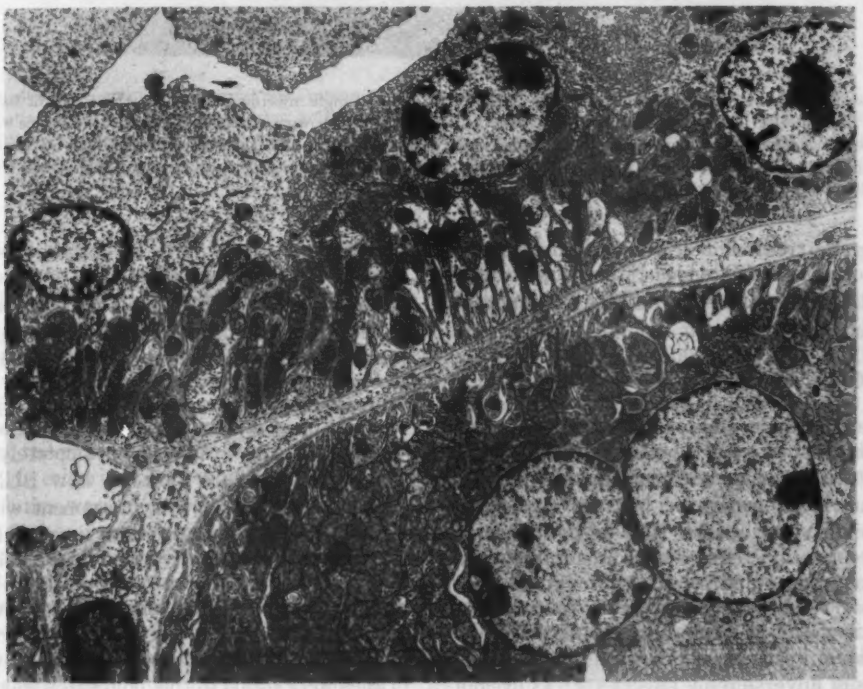

FIGURE 5.—View of cells in the mouse distal renal tubule 8 hrs after a single injection of cytembena at a dose of 100 mg/kg iv. The BUN level has doubled to 32 mg/100 ml. The cell on the left of the photograph shows early cell swelling indicated by decreased cytoplasmic density. A single mitochondrion is undergoing basal swelling with fragmenting cristae. Extensions of this affected cell containing perimitochondrial edema may be seen interdigitating with an adjacent cell. These early changes contrast with the cells which appear to be normal in the lower half of the photograph (× 5400).

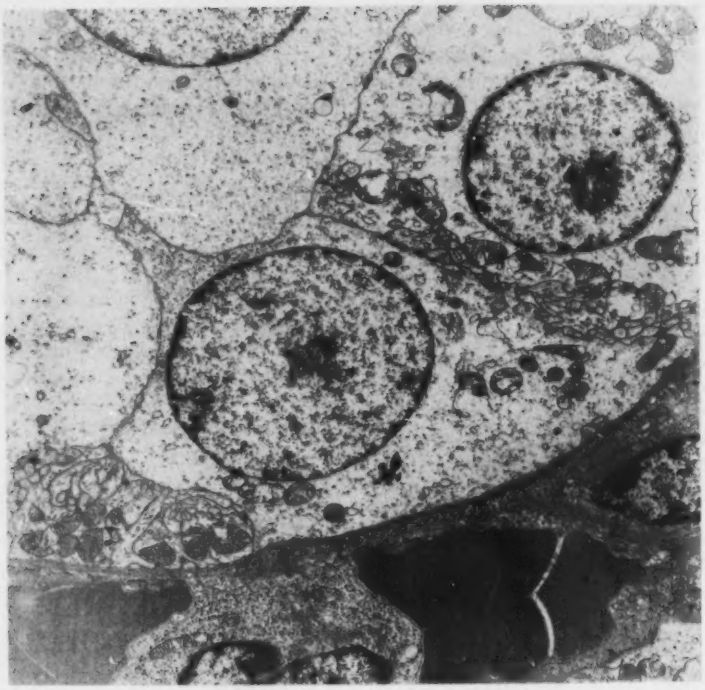

FIGURE 6.—View of cells in the distal renal tubule from a mouse 24 hrs after a single injection of cytembena at a dose of 100 mg/kg iv. The BUN level is 70 mg/100 ml. The epithelial lining cells bordering a capillary are showing extensive degenerative changes with swollen and distorted mitochondria having ruptured limiting membranes and fragmented cristae. Generalized apical swelling is also a common feature. In addition, the nucleus in the upper right has a collection of flocculent electron densities. The capillary endothelial cells show slight cytoplasmic swelling but are otherwise normal (× 5400).

Changing the treatment regimen from single or five daily doses to the extended intermittent schedules did not alter the toxicity patterns which have been described.

## DISCUSSION

Cytembena has undergone extensive clinical trials in Europe as an anticancer agent. In addition to the claims of antineoplastic activity considerable enthusiasm has been generated because significant clinical toxicity was absent. The most commonly reported adverse toxic effects were gastrointestinal signs such as nausea and vomiting. Similar responses were observed in this animal study immediately after injection. This is probably an indirect effect since no gastrointestinal lesions were found at necropsy. Signs of adverse renal effects such as uremia and albuminuria also occurred during several clinical trials but apparently were not serious enough to limit the investigations. The studies described in this report show that renal damage is a serious toxic effect in monkeys at a dosage level which is twice the effective cytotoxic dose as established in rodent studies. Monkeys receiving doses of 25 mg/kg/day of cytembena (300 mg/m$^2$) for 5 days had altered renal function and damage to the tubular epithelium. A similar treatment schedule (150 mg/m$^2$) inhibited the development of transplanted rodent tumors (2). Moreover, complete functional recovery cannot be assured by stopping drug administration since this toxic effect appears to be incompletely reversible. For example, figure 4 shows how BUN levels increased in dogs following treatment with an injection of 100 mg/kg iv. Although elevated BUN values gradually fell and approached the baseline level following drug withdrawal, a significant uremia remained 6 weeks after drug treatment. At necropsy, slightly pitted cortices with shrunken proximal tubules having thickened and wrinkled basement membranes were found in this dog which was examined 6 weeks after treatment. In addition, epithelial cells were hypertrophied, containing basophilic cytoplasm and enlarged nuclei.

These renal changes followed a dose response pattern. The most extensive damage occurred at the

FIGURE 7.—Electron photomicrograph of a proximal renal tubule from the mouse described in fig 6. The cell next to the capillary (upper right) shows characteristic degenerative signs, cytoplasmic swelling, and swollen, distorted mitochondria. The cell on the left side of the photograph shows additional changes which were found only occasionally, swollen endoplasmic reticula (clear, circular spaces) and unidentified, uniformly dark vacuoles (× 5400).

highest toxic dose levels and diminished sharply at descending dosage levels. Furthermore, the type and location of tubules which were affected seemed to have a dose-level relationship.

In both dogs and monkeys necropsied within 24 hours after toxic doses, or in animals that received the lowest toxic dose levels, enough cellular detail remained in the damaged cells to permit a tentative identification of the type of tubules involved. Based upon morphologic criteria it was concluded that the distal renal tubules were the more sensitive structure. The epithelial destruction appeared in both the proximal and distal convoluted tubules at the highest toxic and lethal dosage levels in both dogs and monkeys. However, these changes were found in tubules which were morphologically identified as distal in animals treated at the lowest toxic doses. It should be re-emphasized that this conclusion is based on morphologic destruction such as the size and shape of the lining epithelial cells and the presence or absence of a discernible brush border. A differential analysis of specific tubular function which might precisely pinpoint an impairment in the distal tubules, such as urine acidification or potassium excretion, was not included in this study.

Because of the question of lesion reversibility, ultrastructural changes were examined in mice injected with cytembena (100 mg/kg iv) and killed 8 and 24 hours later. Figure 5 shows the early changes in a cross-sectioned tubule which is identified as distal by the rectilinear arrangement of the mitochondria against the basement membrane and the absence of microvilli. One cell is showing signs of perimitochondrial edema. Next to it is a cell which has undergone a decreased cytoplasmic density indicating early cell swelling. Signs of advanced cellular destruction appeared after 24 hours in the mitochondria which were disarranged and swollen and in some cases ruptured with fragmented cristae (fig 6). These changes must be considered nonspecific since similar renal lesions can be produced by a variety of agents such as irradiation (13), heavy metals, and other organic nephrotoxins (14).

Distal tubular necrosis after treatment with a variety of inorganic agents is a well-established toxic phenomenon which can be readily produced in a

variety of laboratory animals. An organic chemical having the same primary toxic effect is a more unique finding. For example, a review of experimental nephrotoxicity by Zbinden (15) identified 25 organic chemicals which have been shown to destroy renal tubules. In 23 of these examples the proximal tubules were the cells at risk, while one, chlorquinadol, is reported to act exclusively on the distal tubule. Another chemical, amphotericin B, has a proven capacity for injuring both proximal and distal tubules in dogs (16). Subsequently, patients studies with this antibiotic have shown clinical signs, potassium loss, and reduced ability to excrete acid, which suggest the defect is in distal tubular function (17). Cytembena appears not to resemble either of these agents. It has a broader species spectrum of effects than chlorquinadol which is a renal toxin in rodents and hamsters, but not in dogs, cats, and monkeys according to Meier-Ruge (18). Cytembena lacks at least two features of the amphotericin B-induced nephrotoxicity, namely a toxic effect to the glomeruli and the induction of extensive renal calcification (16).

A renal phenomenon such as this raises questions concerning toxic mechanisms. While the data from this study do not conclusively support any hypotheses, several were considered and tested. For example, a vascular etiology seems a remote possibility since all renal vessels were free of thrombi, inflammation, and constrictions. Moreover, the affected renal tissues appeared well oxygenated and without ischemia, calcification, hemoglobin casts, or concurrent infections.

Solubility is an important consideration. A filtered drug would be concentrated in the distal tubules and might exceed its level of solubility in the tubular filtrate. The drug crystals thus formed would be physically damaging to the lining cells. The hematuria which appeared in the treated animals supported this line of reasoning. While crystals were absent in the formalin-fixed specimens of renal tissue taken at necropsy, they might have been lost during histologic processing. Therefore, the investigations described were conducted in two groups of mice which were excreting normal and acidified urine. No foreign crystals were observed by polarized or phase contrast microscopy in any of the treated and uremic animals killed at intervals ranging from 4 to 48 hours. Tubular lesions in a separate group of treated mice were examined further by electron microscopy and found to be free of microcrystals.

Studies of the urinary excretion patterns of cytembena in mice and human volunteers have been reported (19-21) and have shown remarkable species differences. Mice rapidly excreted this compound, ie, 66% within 3 hours. In contrast, only 8% of the injected dose appeared in human urine within 15 hours (20). Because it is a small molecule this compound should be rapidly filtered by the glomeruli. Therefore, to account for this long serum half-life (15 hours), Schuck et al (20) concluded that cytembena must undergo extensive renal reabsorption in humans.

A published report of cytembena toxicity in rats noted only mild renal changes following a dose of 50 mg/kg/day sc for 6 months (2). The results of our study would suggest that there should have been more tubular destruction with this regimen in a susceptible animal. That the rat may be a relatively resistant species was demonstrated by Jelinek (2) in his single-dose toxicity studies which showed a significant difference in LD50 values between rats and mice, 254 versus 100 mg/kg respectively. In our studies the single-dose toxicity in dogs was closer to that seen in the mouse with an LD50 of approximately 100 mg/kg (table 1).

Lymphoid toxicity, which was the second major observation in this study, is also consistent with published observations of the immunosuppressive properties of this agent. Bohunická et al (22) showed a significant blocking activity with cytembena in C3H mice after injections of 40 mg/kg/day for 3 days prior to administrations of antigenic sheep red blood cells. This dose was highly toxic to their animals and induced 60% mortality. Tissue examinations were not included in that report. The present results suggest that this regimen would have caused considerable lymphoid tissue destruction in a susceptible species.

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# Chemotherapy of Transplantable Adenocarcinomas of the Colon in Mice[1,2,3]

J. A. Double[4] and C. R. Ball[5,6]

## SUMMARY

**Transplantable adenocarcinomas of the colon in mice have been developed from primary tumors induced by 1,2-dimethylhydrazine. Two such transplant lines, MAC-13 and MAC-15, have been used to assess the possible value of this type of tumor in chemotherapy screening. A protocol has been established and 11 standard drugs were tested against the two lines. Both tumors show sensitivity which is remarkably similar to that of human large bowel cancer, and MAC-13 would have correctly predicted the activity in man for ten of the 11 drugs. Quantitatively, CCNU, methyl-CCNU, and cyclophosphamide were the most effective drugs. A comparison of the predictive efficiencies of L1210 leukemia, B16 melanoma, and these new tumors as screening systems for colorectal cancer is made and discussed.**

[Cancer Chemother Rep 59:1083-1089, 1975]

One of the major problems of screening for new antitumor agents is that the tests employed are nonspecific, in the same sense that although positive results in current animal systems give some predictability of activity in man, extensive clinical trials are still required to establish which particular human cancers a new drug is effective against. Some solid tumors, such as the B16 melanoma, have been added to the major screening programs in recent years in the hope of identifying drugs with activity against the major solid tumors from among those already selected as active by the primary screens, L1210 and P388 leukemias. Another approach may be to design screening systems capable of selecting drugs with activity against a particular human disease. The availability of new tumor models which are histopathologically related to human colorectal cancer enables this possibility to be put to the test. This paper describes the first phase of an evaluation of two transplantable colon tumor lines in mice as secondary chemotherapy screening systems.

The development of several transplantable adenocarcinomas of the large bowel in mice from primary tumors induced by prolonged administration of 1,2-dimethylhydrazine has been described elsewhere (1). Two such lines have been chosen for appraisal as screening models in this study. Their growth characteristics, tritiated thymidine labelling indices, histopathology (1,2), and some preliminary chemotherapy data have been previously reported (2,3).

## MATERIALS AND METHODS

### Tumor Transplantation

The mouse adenocarcinoma of the colon lines used in this study (MAC-13 and MAC-15) have been previously described and are now in the 20th and 21st transplant generation respectively without any change in growth rate being observed. They were propagated by sc implant of tumor fragments in the flank of NMRI mice in the following way. The tumor was excised from the donor animal and small fragments removed for incubation in media for sterility testing; the remainder was placed in TC199 medium containing streptomycin and penicillin and cut into

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[2]Supported by grants from the Yorkshire Cancer Research Campaign and the Medical Research Council.

[3]5-Fluorouracil, methotrexate, BCNU, CCNU, methyl-CCNU, mitomycin C, adriamycin, and vincristine were supplied by the Division of Cancer Treatment, National Cancer Institute. Chlorambucil and melphalan were supplied by Burroughs Wellcome Ltd, Research Triangle Park, NC. Cyclophosphamide was supplied by Ward, Blenkinsop & Co, Ltd, Wembley, Middlesex, Great Britain.

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[6]We thank Ms. Jill Rothwell and Ms. Merry Hutchinson for expert technical assistance and Dr. M. J. Fulker for carrying out computer analyses.

small fragments approximately 1 × 2 mm in size. Fragments were implanted in the flank using a trocar. MAC-13 was routinely passaged in female mice and MAC-15 in males.

### Mice

The NMRI mouse strain used in these experiments is a pure line, bred in our own laboratories from a stock originally obtained from the Medical Research Council Laboratory Animal Centre. The animals were fed on Oxoid pasteurized breeding diet and water ad libitum.

### Chemotherapy Testing

Earlier experiments had been carried out to establish that the take rate by trocar fragments approached 100% and that the growth rates as determined by 3-4-day caliper measurement were consistent and, therefore, the lines appeared to be suitable models for chemotherapy (3).

Measurement of the growth delays induced by drugs could have been used as a method of assessing the effects, but this is somewhat subjective and is certainly time-consuming. Survival time assays, although more objective, were impractical because large tumors tended to ulcerate through the skin, with consequent random non-tumor-associated deaths. It was, therefore, decided to establish a protocol based on tumor weight measurements.

The use of fragment implants quite naturally produced a considerable range of tumor sizes, since it is hard to accurately control either tumor load or fragment size. The use of a tumor homogenate did not narrow the range of tumors produced and since it was felt that such a procedure might encourage dedifferentiation, it was not pursued further. The system is at present under further development and attempts at stepwise reductions are being investigated in order to make it more amenable for routine screening.

With these factors in mind, it was decided that in this study only established tumors (approximately 5 × 5 mm) would be treated so that their size at the time of treatment was known and could be controlled. A procedure was devised to select animals with tumors in a certain size range based on tumor volumes calculated from caliper measurements in the standard way (4).

A typical size distribution at the time of selection is shown in table 1. Using such a histogram, a group of tumors was selected in which the size range was less than fivefold (*see* "Protocol for Chemotherapy Tests" for details) and these animals were used for a chemotherapy test. It was assumed (and later proved) that the weight range of control tumors at the time of evaluation of the chemotherapy test would also not exceed fivefold. This being so, and using 15 animals in the control group, a treated group was expected to be significantly different from a control group only if the mean %T/C was < 35% (ie, > 65% tumor inhibition).

TABLE 1.—Tumor selection procedure

| Group | Tumor size range ($mm^3$) | No. of animals in range | Selection |
|---|---|---|---|
| A | <40 | 8 | Rejected |
| B | 40-60 | 32 | |
| C | 60-90 | 27 | 79* |
| D | 90-135 | 17 | 48 |
| E | 135-200 | 3 | |
| F | 200-300 | 1 | |
| G | >300 | 0 | Rejected |
| Total | | 88 | |

*Groups B, C, D, and E had the largest number and were randomized and used in the chemotherapy test.

### Morphometric Analysis of MAC-13

The tumor line MAC-13 is characterized by large cysts containing mucin-like material (1) and it is not possible to excise the tumor without rupturing some of the cysts and losing the fluid. For this reason the following technique was used. Excised tumors often weighed some 25% less than the difference in weight between the carcass with and without the tumor and it was clear from histologic sections that 30%-40% of tumor volume was occupied by cystic fluid. It was clearly possible that false-positive chemotherapy results might be obtained if a drug merely reduced the amount of fluid produced within the tumor. Histologic sections (hematoxylin and eosin stain) of tumors from treated and control groups were thus compared to determine whether results could be invalidated in this way. The section was projected onto white paper using a Zeiss Projection Microscope and the tumor cross sectional outline drawn on the paper. The outline of cystic spaces was drawn on a second piece of paper. These two drawings were analyzed by a Quantimet Image Analyzing Computer to arrive at a figure for the percentage of cross sectional area of the tumor which was cystic space. No statistically significant differences were found between the treated tumors and the controls where the cystic space was 32.6% (SE 47) of the area.

## PROTOCOL FOR CHEMOTHERAPY TESTS

*Tumor selection.*—Tumors had grown to a suitable size for use some 2-3 weeks after transplantation. At this time each tumor was measured with calipers, tumor volume was calculated, the animals were sorted according to tumor size, and a histogram was plotted as shown in table 1. Animals bearing tumors $< 40$ or $> 300$ mm$^3$ were rejected. Animals in groups B, C, D, and E or in groups C, D, E, and F (whichever was the greater number) were selected for the chemotherapy test. The starting size range was thus either 40-200 or 60-300 mm$^3$. Eighty percent of the animals were accepted for use provided the selection was carried out at a time when the median of tumor size was in group C, D, or E. The selected animals were randomized into groups for the test so that the control group contained 15 animals and the treated groups each contained eight. When there were more than six treated groups in one test the size was reduced to seven.

*Drug administration.*—All treatments in this study were by ip injection and the control groups received an appropriate solvent injection. Three dose tests were carried out in all experiments, with 1.5-fold spaced doses, in such a way that the top dose was expected to have some toxicity.

*Toxicity.*—All animals were weighed on Day 5 after treatment and any drug that induced weight loss was recorded. Deaths were recorded daily throughout the experiment. The LD10s of the drugs used in this study are shown in table 2 and were derived from probit plots of mortality data.

*Evaluation.*—In order to compare the results from the two tumor lines the evaluation day was chosen to be at a time when controls had increased tenfold in volume from the start of treatment. On the appropriate day (Day 15 for MAC-13 and Day 22 for MAC-15), the animals were killed and weighed, the tumor was carefully excised, and the carcass without the tumor was reweighed. The tumor weight was thus determined by the difference. This method was necessary because the high fluid content of MAC-13 (described above) made it impractical to obtain a meaningful measurement by weighing the excised tumor.

*Statistics.*—The results from treated groups in which either $> 25\%$ of the animals died or in which there was a $> 15\%$ weight loss on Day 5 were rejected (these were determined to be toxic groups). The remaining tumor weights were subjected to analysis of variance (5). When this indicated a significantly different group at $P < 0.05$, 95% and 99% confidence limits were calculated for all groups. In the treated groups where these limits did not overlap with those of the control group the results were regarded as statistically significant and their significance at the two levels of 95% and 99% are shown in tables 3 and 4.

## CHEMOTHERAPY RESULTS

The results of the chemotherapy tests according to the above-mentioned protocol are listed in table 3

TABLE 2.—Drug toxicity in tumor-bearing NMRI mice

| Drug | Schedule | LD50 (mg/kg) |
|---|---|---|
| 5-FU | Single | 205 |
| 5-FU | qd× 5 | 44 |
| Cyclophosphamide | Single | 550 |
| Methotrexate | qd × 5 | 4.2 |
| BCNU | Single | 70 |
| CCNU | Single | 60 |
| Methyl-CCNU | Single | 42 |
| Chlorambucil | Single | 38 |
| Mitomycin C | Single | 7 |
| Adriamycin | Single | 11 |
| Melphalan | Single | 15 |
| Vincristine | Single | 3.2 |

for MAC-13 and in table 4 for MAC-15 for 11 standard drugs. Examination of the results in these tables shows that even though significant effects are seen with about half of the drugs tested, both tumors are relatively insensitive to chemotherapy. Therapeutic indices are low with significant effects only being seen close to the maximum tolerated dose. No attempt has been made in this study to optimize drug schedules. 5-Fluorouracil (5-FU) therapy was not very effective against either tumor, and the only effects seen were on the single dose schedule. Cyclophosphamide and the nitrosoureas were the best drugs in both tumors and the only drugs to produce effects at more than one dose level. For both tumor lines the order of effectiveness of the nitrosoureas was CCNU > methyl-CCNU > BCNU, and BCNU was completely ineffective against MAC-15. Mitomycin C was effective against both lines but only at the maximum tolerated dose and chlorambucil had similar activity against MAC-15 only. Methotrexate, adriamycin, melphalan, and vincristine were inactive in both systems.

It was noted during this study that once the tumors were established and were of the appropriate size range for chemotherapy (ie, 40-200 $mm^3$), therapeutic effects were almost identical on large and small tumors. However, preliminary experiments with 5-FU have shown that treatment 2 days after transplantation produced "cures."

## DISCUSSION

The objectives of this study were to examine the chemotherapy sensitivity of the two transplantable tumor lines derived from colorectal cancer in mice and to compare these with results of chemotherapy for human colon cancer. Specifically, we wanted to find out whether this model system might have any predictive value as a secondary screen for agents against human colorectal cancer. The problems of evaluation of a new potential screening system have recently been reviewed by Carter (6). The screening system in use today, and principally the L1210 leukemia screen, has yielded some 30 compounds of proven clinical value. There is no reason to suppose, however, that the L1210 leukemia in particular, with its very "non-human" characteristics, should have any special predictive value for any particular neoplastic disease.

The B16 melanoma, with several growth characteristics more similar to those of human solid tumors, has been introduced as a secondary screen in an attempt to include better agents for such tumors.

In attempting to show the tumor type specificity of a new model, comparisons must be made not only with the human disease but also with existing model systems. In man, the three nitrosoureas (BCNU, CCNU, and methyl-CCNU), mitomycin C, and 5-FU have proven therapeutic value against colorectal cancer, and there is evidence of activity, which requires further substantiation, for cyclophosphamide and methotrexate (7). The pattern of drug sensitivity seen in this study is similar, the primary exception being the insensitivity of the mouse models to methotrexate. There is some response to 5-FU and mitomycin C, but the nitrosoureas and cyclophosphamide are the most effective drugs. CCNU and methyl-CCNU have similar activity but are superior to BCNU. Adriamycin, a drug with significant therapeutic value against many solid tumors but not against those of the large bowel in man, was also ineffective on either of the model colon cancers in mice (8).

The ability of the MAC-13 and MAC-15 tumor lines to pick out drugs with a high potential for activity against human colon cancer has been compared to that achieved by the standard L1210 leukemia and B16 melanoma screens (table 5). It can be seen that the MAC tumor lines are more efficient screens for predicting drugs active against colon cancer in man. Five false predictions out of 11 drugs would have been made if the B16 melanoma had been used to predict activity in large bowel cancer. The L1210 leukemia proves more accurate but it is mistaken on the false-positive side, whereas the MAC tumor lines tend to err on the false-negative side. MAC-13 gave only one incorrect result in terms of predictive efficiency which was a false negative. It should be pointed out that all the results have been substantiated on at least three occasions, but with the exception of 5-FU and methotrexate all drugs have been given only in single dose schedules (tables 3 and 4). The effects of dose scheduling and combination therapy have not yet been investigated. Although direct comparisons between different screening systems are often difficult because they use different criteria and end points to determine drug effects (eg, survival time in the B16 melanoma and tumor weight in the MAC system), a comparison of the predictive efficiency of the two systems (if used for human colorectal cancer) shows the possible advantages of the MAC system over B16 melanoma. This is illustrated diagrammatically in figure 1. Each tumor is represented by a circle and the drugs that show activity are placed within the circle.

Only 11 of the 24 standard drugs have been tested so far (and these only by single dose schedules), but results indicate that the tumor lines may have some value as secondary screening systems in the search for drugs active against large bowel cancer. Extending the type of analysis mentioned above to the remaining standard drugs and comparisons of the

TABLE 3.—Chemotherapy test results for MAC-13

| Drug | Schedule | Vehicle | Dose (mg/kg) | Fraction LD10 | No. of survivors/ No. of animals treated | T/C (%) | Inhibition (%) |
|---|---|---|---|---|---|---|---|
| 5-FU | Single | Saline | 320 | — | 2/8 | — | — |
| | | | 214 | 1.0 | 7/8 | 34* | 66* |
| | | | 142 | 0.66 | 8/8 | 39* | 61* |
| | | | 60 | — | 0/8 | — | — |
| | qd × 5 | Saline | 40 | 0.91 | 8/8 | 54 | 46 |
| | | | 27 | 0.61 | 8/8 | 69 | 31 |
| Cyclophosphamide | Single | Saline | 450 | 0.82 | 7/7 | — | — |
| | | | 300 | 0.54 | 7/7 | 18† | 83† |
| | | | 200 | 0.36 | 7/7 | 33* | 67* |
| Methotrexate | qd × 5 | Saline | 6.0 | — | 4/8 | — | — |
| | | | 4.0 | 0.95 | 7/8 | 89 | 11 |
| | | | 2.7 | 0.64 | 8/8 | 104 | 0 |
| BCNU | Single | 10% ethanol, peanut oil | 90 | — | 1/8 | — | — |
| | | | 60 | 0.85 | 8/8 | 29* | 71* |
| | | | 40 | 0.57 | 8/8 | 56 | 44 |
| CCNU | Single | 10% ethanol, peanut oil | 90 | — | 1/8 | — | — |
| | | | 60 | 1.0 | 7/8 | 4† | 96† |
| | | | 40 | 0.67 | 8/8 | 2† | 98† |
| Methyl-CCNU | Single | 10% ethanol, peanut oil | 40 | 0.95 | 8/8 | — | — |
| | | | 27 | 0.64 | 8/8 | 21* | 79* |
| | | | 18 | 0.43 | 8/8 | 61 | 39 |
| Mitomycin C | Single | Saline | 10 | — | 0/8 | — | — |
| | | | 6.7 | 0.96 | 8/8 | 25* | 75* |
| Adriamycin | Single | Saline | 9 | 0.82 | 7/7 | — | — |
| | | | 6 | 0.55 | 7/7 | 88 | 12 |
| | | | 4 | 0.37 | 7/7 | 120 | 0 |
| Melphalan | Single | Propylene glycol buffer | 12 | 0.8 | 7/7 | — | — |
| | | | 8 | 0.53 | 7/7 | 45 | 55 |
| | | | 5.6 | 0.37 | 7/7 | 79 | 21 |
| Chlorambucil | Single | 10% ethanol, peanut oil | 45 | — | 3/6 | — | — |
| | | | 30 | 0.79 | 6/6 | 48 | 52 |
| Vincristine | Single | Saline | 4.0 | — | 1/8 | — | — |
| | | | 2.7 | 0.84 | 8/8 | 44 | 56 |
| | | | 1.8 | 0.56 | 8/8 | 87 | 13 |

*Significant tumor inhibition by analysis of variance at $0.05 > P > 0.01$.
†Significant inhibition at $P < 0.01$.

TABLE 4.—Chemotherapy test results for MAC-15

| Drug | Schedule | Vehicle | Dose (mg/kg) | Fraction LD10 | No. of survivors/ No. of animals treated* | T/C (%) | Inhibition (%) |
|---|---|---|---|---|---|---|---|
| 5-FU | Single | Saline | 320 | — | 3/8 | — | — |
| | | | 214 | 1.0 | 6/8 | 37* | 63* |
| | | | 142 | 0.66 | 8/8 | 80 | 20 |
| | | | 45 | 1.0 | 5/7 | 62 | 38 |
| | qd × 5 | Saline | 30 | 0.68 | 7/7 | 102 | 0 |
| | | | 20 | 0.46 | 7/7 | 108 | 0 |
| Cyclophosphamide | Single | Saline | 600 | — | 6/8 | — | — |
| | | | 400 | 0.73 | 8/8 | 14† | 86† |
| | | | 266 | 0.48 | 8/8 | 24† | 76† |
| Methotrexate | qd × 5 | Saline | 6.0 | — | 3/8 | — | — |
| | | | 4.0 | 0.95 | 8/8 | 68 | 32 |
| | | | 3.0 | 0.71 | 8/8 | 75 | 25 |
| BCNU | Single | 10% ethanol, peanut oil | 80 | — | 1/8 | — | — |
| | | | 56 | 0.80 | 8/8 | 57 | 43 |
| CCNU | Single | 10% ethanol, peanut oil | 60 | 1.0 | 7/8 | — | — |
| | | | 40 | 0.67 | 8/8 | 13† | 87† |
| | | | 27 | 0.45 | 8/8 | 43† | 57† |
| Methyl-CCNU | Single | 10% ethanol, peanut oil | 45 | — | 1/8 | — | — |
| | | | 30 | 0.71 | 8/8 | 34† | 66† |
| | | | 20 | 0.48 | 8/8 | 45† | 55† |
| Mitomycin C | Single | Saline | 10 | — | 3/8 | — | — |
| | | | 6.6 | 0.94 | 8/8 | 44* | 56* |
| Adriamycin | Single | Saline | 12 | — | 8/8 | — | — |
| | | | 8 | 0.73 | 8/8 | 76 | 24 |
| | | | 5.3 | 0.48 | 8/8 | 80 | 20 |
| Melphalan | Single | Propylene glycol buffer | 18 | — | 2/7 | — | — |
| | | | 12 | 0.80 | 7/7 | 81 | 19 |
| | | | 8 | 0.44 | 7/7 | 94 | 16 |
| Chlorambucil | Single | 10% ethanol, peanut oil | 45 | — | 7/8 | — | — |
| | | | 30 | 0.71 | 8/8 | 50* | 50* |
| | | | 20 | 0.48 | 8/8 | 62 | 48 |
| Vincristine | Single | Saline | 4.0 | — | 1/8 | — | — |
| | | | 2.6 | 0.81 | 8/8 | 107 | 0 |
| | | | 1.8 | 0.43 | 8/8 | 86 | 14 |

*Significant tumor inhibition by analysis of variance at $0.05 > P > 0.01$.
†Significant inhibition at $P < 0.01$.

TABLE 5.—Efficiency of various screening systems if used to predict activity in human colorectal cancer of 11 standard drugs

| System | No. of — | | | |
|---|---|---|---|---|
| | True positives | True negatives | False positives | False negatives |
| L1210 leukemia | 7 | 2 | 2 | 0 |
| B16 melanoma | 5 | 1 | 3 | 2 |
| MAC-13 | 6 | 4 | 0 | 1 |
| MAC-15 | 5 | 3 | 1 | 2 |

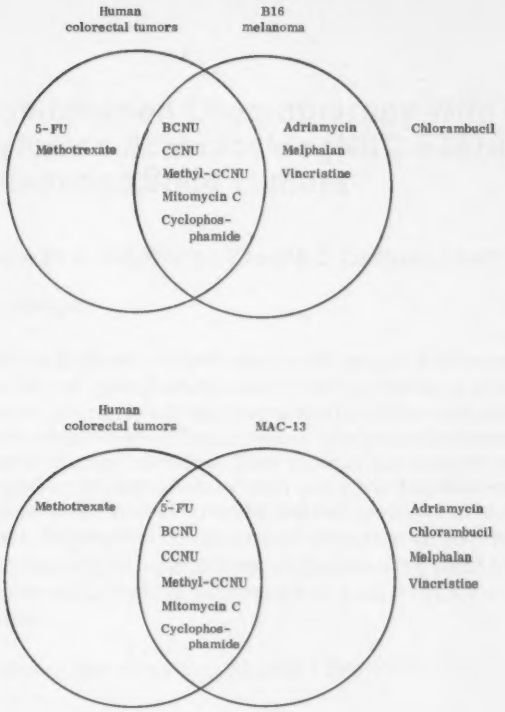


FIGURE 1.—Illustration of the predictive efficiency of B16 melanoma and MAC-13.

effects of new drugs with their effects in phase II clinical trial would establish whether a screening system of this type would be superior to those used currently.

## LIST OF COMPOUNDS

**Adriamycin:** NSC-123127; CAS reg. No. 23214-92-8
**BCNU:** NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
**CCNU:** NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
**Chlorambucil:** NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-
**Cyclophosphamide:** NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
**5-Fluorouracil:** NSC-19893; CAS reg. No. 51-21-8; 5-FU
**Melphalan:** NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-
**Methotrexate:** NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
**Methyl-CCNU:** NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
**Mitomycin C:** NSC-26980; CAS reg. No. 50-07-7
**Vincristine:** NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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# Combination Chemotherapy With Cyclophosphamide (NSC-26271), Cytosine Arabinoside (NSC-63878), and Methotrexate (NSC-740) in Advanced Solid Tumors[1,2]

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## SUMMARY

**Forty patients with advanced solid tumors of diverse primary sites received a combination of cyclophosphamide (1 g/m$^2$), cytosine arabinoside (300 mg/m$^2$), and methotrexate (80 mg/m$^2$) given intermittently at 2-3-week intervals. Eight of the 40 patients received citrovorum factor rescue. The major limitation of therapy was suppression of bone marrow elements. Only minimal nonhematologic toxicity was encountered. Granulocytes appeared the most sensitive. The first course of treatment produced median nadir granulocyte and platelet counts of 1200 and 100,000 cells/mm$^3$ respectively. Subsequent courses were tolerated with no evidence of increasing myelosuppression. Objective antitumor responses were noted in five of 16 patients with lung cancer and in eight of 14 women with breast cancer with a median duration of 8 months.**

[Cancer Chemother Rep 59:1091-1096, 1975]

The use of drugs in combination therapy has been a major thrust in cancer chemotherapy in the last decade (1,2). Significant improvements in the number and extent of tumor regressions and prolongations in survival have been achieved through such therapy (3-5) as compared to other reported benefits of single-agent therapy in similar diseases.

The potential for combination chemotherapy has been increased by the development of new effective antineoplastic drugs and an appreciation for tumor cell kinetics which permits more optimal scheduling of drugs in relation to tumor growth and host toxicity. Low growth fractions in situations with large body burdens of tumor cells result in diminished sensitivity to cell cycle specific agents (6,7); combinations of cell cycle specific and cell cycle nonspecific drugs may result in greater cell kill (8). Using this rationale, we designed a treatment program in acute leukemia using cyclophosphamide as the cell cycle nonspecific agent and a combination of methotrexate and cytosine arabinoside as the cell cycle specific components (9). The current study examined this schedule in patients with solid tumors to quantitate the toxicity of these agents in patients with normal bone marrow as well as to gauge therapeutic effectiveness.

## MATERIAL AND METHODS

The initial evaluation of patients included a complete history and physical examination, appropriate roentgenographic studies (metastatic bone survey and bone scan in some patients), liver function studies, electrocardiogram, blood urea nitrogen, serum creatinine, and a complete blood cell count. A bone marrow aspiration and biopsy were done on some patients to determine marrow involvement with disease or marrow reserve following aggressive prior therapy. Complete blood cell counts were obtained at weekly intervals. Granulocyte counts were determined from the percentage of neutrophils and bands in the white blood cell counts. Patients with abnormal creatinine levels were excluded from the study.

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[2]Supported in part by Public Health Service grants CA-08341 and CA-95138 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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[7]We are grateful to the nurses of the Clinical Research Center, especially Head Nurse Mrs. Shirley Blood, RN, and our Special Procedures nurses, Mrs. Knobf, RN, and Mrs. Johnson, RN, for their assistance in the care of these patients.

TABLE 1.—Disease categories and therapeutic responses in patients treated with CAM and CAM-CF

| Tumor type | No. of evaluable patients treated | No. of courses | No. of responses* | | |
|---|---|---|---|---|---|
| | | | PR | SD | PD |
| Lung | 16 | 52 | 5 (5,5,5,4,2) | 2 (5,2) | 9 |
| Breast | 14 | 73 | 8 (15,11,10,9,7,4,4,2) | 2 (2,2) | 4 |
| Anaplastic carcinoma with unknown primary | 4 | 18 | 3 (3,2,2) | — | 1 |
| Bladder | 2 | 7 | — | 2 | — |
| Cervix | 1 | 3 | — | 1 | — |
| Penis | 1 | 3 | — | — | 1 |
| Fibrosarcoma | 1 | 2 | — | — | 1 |
| Olfactory neuroepithelioma | 1 | 4 | — | 1 | — |
| Total | 40 | 162 | 16 | 8 | 16 |

*See text for definition of responses. Nos. in parentheses indicate duration of response in months.

Other laboratory studies were repeated during the course of therapy as clinically indicated.

Forty patients with advanced malignancies of various origin were treated (table 1). Six of the 40 patients had received no drug or radiation therapy before starting the cyclophosphamide, cytosine arabinoside, and methotrexate (CAM) regimen. Fourteen of 16 patients with lung cancer had received prior radiotherapy in the dose range of 2700-4000 roentgens to the thorax lesion and one patient had additional radiotherapy to the brain. In addition, one patient had received mechlorethamine and another patient who initially was thought to have adrenocortical carcinoma received mitotane for treatment of adrenal involvement before CAM therapy was started. All of the 14 patients with breast cancer had had prior hormonal manipulation and had either initially failed or had become refractory to that therapy before cytotoxic therapy with CAM. In addition, 5-fluorouracil had been administered to seven patients, alkylating agents (cyclophosphamide, chlorambucil) to three, and vincristine to one; three had had no prior chemotherapy. The metastatic sites of involvement among the patients with breast cancer were: skin (either chest-wall recurrence or distant site) (seven patients); bone (six patients); parenchymal lung disease (three patients); liver (three patients); malignant pleural effusion (two patients); and brain (two patients). The patient with cancer of the cervix had received prior radiotherapy as well as bleomycin and CCNU. The two patients with cancer of the bladder had failed to be controlled with radiotherapy and adriamycin, and the patient with cancer of the penis had failed with bleomycin and a penectomy. The four patients with unknown primary sites had received no therapy prior to CAM treatment.

Objective measures of the disease were obtained by radiographic or radioisotopic methods or by direct measurements where applicable. Measurements in two dimensions were taken before therapy and at intervals during therapy. The responses were classified according to objective regressions of measurable disease (10). A complete objective response (CR) was considered to be the disappearance of all measurable disease. A partial remission (PR) was > 50% regression of all measurable disease as determined by the product of the two widest diameters for a period > 1 month. Stable disease (SD) was the absence of any change in measurable disease and progressive disease (PD) was the development of new lesions or an increase by > 50% of target lesions while the patient was receiving an adequate course of therapy.

Therapy consisted of rapid infusion of cyclophosphamide (1 g/m$^2$) followed in 30 minutes by cytosine

arabinoside (300 mg/m$^2$), and followed immediately by the initial dose of methotrexate. Methotrexate was given iv in three equal doses at 8-hour intervals and the total 24-hour dose was 80 mg/m$^2$. In the interest of safety, the initial eight patients treated in the study received citrovorum factor given orally at a dose of 25 mg every 6 hours for 24 hours with the first dose given 8 hours after the last dose of methotrexate therapy. The treatment was repeated every 2-3 weeks depending on the recovery of hematopoietic function. Provisions were made to reduce the dosage of cyclophosphamide by 25%-50% in subsequent courses for any patients with profound granulocytopenia or delayed recovery of marrow function. A minimum of two courses of therapy was given to each patient. Thereafter, evidence of progression of disease prompted a change of therapy.

## RESULTS

### Toxic Effects

Nonhematologic toxic effects were generally mild. Most patients had nausea and vomiting for several hours after treatment was started. Alopecia and moderate stomatitis each occurred in six patients. The major toxic effect was hematologic. There were no drug-related deaths. Tables 2 and 3 examine the effects of citrovorum factor rescue on the first and subsequent doses of the CAM regimen (CAM-CF) in eight patients receiving 29 courses of treatment. Therapy with CAM-CF resulted in median nadir granulocyte and platelet counts of 2000 and 255,000 cells/mm$^3$ respectively after the first course. Subsequent courses yielded median nadir granulocyte and platelet counts of 2600 and 310,000 cells/mm$^3$ respectively. Except for one patient, patients receiving CAM-CF tolerated multiple courses of therapy without reduction in drug dosage. Although the patient numbers are small, citrovorum factor appears to have favorably affected hematopoietic function after both the initial and subsequent courses of therapy.

Hematologic toxicity also appears to be tolerable in the majority of patients who did not receive citrovorum factor rescue. Table 2 shows the effect of the first course of CAM treatment on the hematocrit, granulocyte, and platelet counts. There was a median nadir granulocyte count of 1200 cells/mm$^3$ and a moderate suppression of platelet function with a median nadir of 180,000 cells/mm$^3$. Suppression of the hematocrit level was not significant since it was not greater than 5 vol %. The greatest degree of suppression (nadir value) occurred on the 10th or 11th day after the therapy ended after which hematologic values gradually rose to pretreatment values.

Table 3 summarizes the effect of subsequent courses of CAM treatment on hematopoietic function. The total number of courses per patient ranged from two to 11 with a median of three courses of treatment per patient. The time to reach the nadir and nadir values were also not significantly different from the results following the first course of treatment.

Of the 40 patients, six had significant pancytopenia attributable to drug treatment as manifested by suppression of hematocrit > 5 vol %, of the granulocyte count to < 600 cells/mm$^3$, and of the platelet count to < 100,000 cells/mm$^3$. All six patients were in the CAM group. The median duration of cytopenia for these patients was 28 days. The drug dose was subsequently reduced in the patients who developed cytopenia lasting > 3 weeks after the first course,

TABLE 2.—Hematologic suppression after first course of treatment

| | Regimen | | | | | |
|---|---|---|---|---|---|---|
| | CAM* | | | CAM-CF* | | |
| | Hematocrit value (vol %) | Granulocyte count (cells × 10$^3$/mm$^3$) | Platelet count (cells × 10$^3$/mm$^3$) | Hematocrit value (vol %) | Granulocyte count (cells × 10$^3$/mm$^3$) | Platelet count (cells × 10$^3$/mm$^3$) |
| Pretreatment value | | | | | | |
| Median | 37 | 5.0 | 280 | 38 | 4.8 | 360 |
| Range | 26-48 | 1.8-15.6 | 75-600 | 31-48 | 3.4-6.1 | 220-440 |
| Nadir value | | | | | | |
| Median | 32 | 1.2 | 180 | 35 | 2.0 | 255 |
| Range | 20-41 | 0.01-7.2 | 64-880 | 31-40 | 1.1-2.8 | 180-330 |

* See text for definition of regimen.

TABLE 3.—Hematologic suppression after subsequent courses of treatment

| | Regimen | | | | | |
|---|---|---|---|---|---|---|
| | CAM* | | | CAM-CF* | | |
| | Hematocrit value (vol%) | Granulocyte count (cells × $10^3$/mm$^3$) | Platelet count (cells × $10^3$/mm$^3$) | Hematocrit value (vol %) | Granulocyte count (cells × $10^3$/mm$^3$) | Platelet count (cells × $10^3$/mm$^3$) |
| Precourse value | | | | | | |
| Median | 34 | 4.9 | 300 | 34 | 4.7 | 375 |
| Range | 26-41 | 1.5-19.2 | 86-870 | 27-43 | 2.7-6.8 | 240-600 |
| Nadir value | | | | | | |
| Median | 32 | 1.7 | 180 | 33.5 | 2.6 | 310 |
| Range | 20-41 | 0.11-5.3 | 20-800 | 24-40 | 0.7-4.6 | 180-520 |

*See text for definition of regimen.

four of whom had had previous extensive radiotherapy. Five of these six patients had an extensive tumor burden, three with bone involvement. Three of the six patients had nadir granulocyte counts <100 cells/mm$^3$ and two had nadir platelet counts < 50,000 cells/mm$^3$. Only one patient had both granulocyte and platelet counts of < 100 and 50,000 cells/mm$^3$ respectively. This patient who had breast cancer and extensive bony metastases with prior radiotherapy to the chest wall manifested the most severe granulocytopenia after the first course. Despite a normal pretreatment granulocyte count, her nadir count was 10 cells/mm$^3$ on Day 11 after treatment with the standard dose of drugs. She had an infectious process requiring hospitalization. She responded well to antibiotic therapy, her granulocyte count rose to normal levels in 21 days, and she tolerated another course of attenuated dose of treatment. Another patient with advanced breast cancer and bone metastases developed nadir granulocyte and platelet counts of 110 and 20,000 cells/mm$^3$ respectively after the 7th course of drug treatment. She had no significant infection or bleeding, marrow recovery occurred, and she eventually tolerated more courses of attenuated dose of drug treatment. She had a remission lasting 15 months after the start of CAM treatment with subjective benefit and radiographic improvement.

### Tumor Response

Table 1 lists the variety of tumor types treated with this combination regimen and the responses observed. No complete remissions were observed. Eight of 14 patients with metastatic cancer of the breast had subjective benefit and objective decreases in tumor size with a median duration of 8 months. Two additional patients had stable disease for 2 months each. Five of 16 patients with advanced cancer of the lung had subjective benefit and favorable measurable objective changes, four of whom had changes lasting > 1 month. Four of these responses were in six patients with oat cell carcinoma. The fifth patient who showed an objective response had the adenocarcinoma cell type. The two patients with stable disease had oat cell carcinoma and adenocarcinoma respectively. No objective responses were observed in the following patients with other cell types: anaplastic large cell carcinoma (four patients), adenocarcinoma (three patients), oat cell carcinoma (one patient), and alveolar cell carcinoma (one patient). Insufficient numbers of patients were entered with tumors of other sites to determine response rates. However, transient responses were seen in patients with cancer of the cervix, bladder, and olfactory neuroepithelioma, and in anaplastic carcinoma of unknown primary. In six patients dose attenuation in the course of therapy did not appear to be a significant factor in the response pattern.

Table 4 shows the responses seen in the eight patients who received CAM-CF. Two patients with oat cell carcinoma of the lung showed objective responses.

## DISCUSSION

It has been shown in studies of late stage L1210 leukemia that the addition of cyclophosphamide to the known synergistic combination of methotrexate and cytosine arabinoside (11-13) yields additive and perhaps synergistic effects.[8] Based upon these studies we have designed a protocol consisting of a predominantly cell cycle nonspecific agent (cyclophos-

[8]Skeel RT, Capizzi R, and Bertino JR. Unpublished data.

TABLE 4.—Tumor responses in patients treated with CAM-CF*

| Tumor type | No. of patients | No. of responses† | | |
|---|---|---|---|---|
| | | PR | SD | PD |
| Lung | | | | |
| Oat cell | 3 | 2 (5,4) | — | 1 |
| Adenocarcinoma | 2 | — | — | 2 |
| Anaplastic large cell | 1 | — | — | 1 |
| Olfactory neuroepithelioma | 1 | — | 1 | — |
| Penis | 1 | — | — | 1 |
| Total | 8 | 2 | 1 | 5 |

*See text for definition of regimen.

†See text for definition of responses. Nos. in parentheses indicate duration of disease in months.

phamide) and two cell cycle specific agents (cytosine arabinoside and methotrexate). Each one of these three drugs has known antineoplastic activity against the leukemias and a variety of solid tumors and each is myelotoxic (14).

The data presented herein indicate that the greatest toxic effect of CAM is on granulopoiesis with less effect on megakaryopoiesis and erythropoiesis respectively. The use of CAM in pulses, with each course given after marrow recovery, has resulted in no appreciable prolonged suppression of marrow function in the majority of patients. Based on the experience with the single patient who had breast cancer, extensive bony metastases, and prior radiotherapy but who had marked myelosuppression on CAM even though pretreatment blood counts were normal, we suggest that patients with extensive bony disease and/or prior extensive radiotherapy begin on an attenuated dose irrespective of pretreatment granulocyte counts.

The lack of additive marrow toxicity in these patients treated with short-term (24-hour) drug combinations is consistent with the concepts Bruce et al (15,16) have derived from animal studies, ie, a significant proportion of marrow stem cells are not "in cycle" and are therefore spared by intensive short-term treatment.

CAM treatment did not appear to significantly improve objective responses over single agents in patients with lung cancer. The overall response rate of 30% is not significantly superior to cyclophosphamide alone (28%) (17) or methotrexate alone (24%) (17). The median survival time of 3.6 months for CAM is also not noticeably different from that for cyclophosphamide alone (2.7 months) or methotrexate alone (2.3 months) (17). Like the responses to cyclophosphamide (50%) and methotrexate (50%) (18), the most frequent responses to CAM are seen in patients with oat cell carcinoma of the lung. Further studies of this combination therapy alone and with additive agents or radiotherapy in oat cell carcinoma are probably warranted.

Eight of 14 patients with disseminated cancer of the breast did achieve significant objective responses. Further studies of this combination in breast cancer are also indicated. Since additional effective agents are now available for treatment of this disease (adriamycin, 5-fluorouracil, and vincristine) (19), the opportunity also exists for the use of this program in sequence or in combination with these drugs.

## LIST OF COMPOUNDS

**Adriamycin:** NSC-123127; CAS reg. No. 23214-92-8

**Bleomycin:** NSC-125066; [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate

**CAM** = cyclophosphamide, cytosine arabinoside, and methotrexate

**CAM-CF** = cyclophosphamide, cytosine arabinoside, methotrexate, and citrovorum factor

**CCNU:** NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

**Chlorambucil:** NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[p-[bis (2-chloroethyl) amino]phenyl]-

**Citrovorum factor:** NSC-3590; CAS reg. No. 6035-86-5; glutamic acid, *N*-[p-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt; leukovorin

**Cyclophosphamide:** NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

**Cytosine arabinoside:** NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; arabinosyl cytosine

**5-Fluorouracil:** NSC-19893; CAS reg. No. 51-21-8

**Mechlorethamine:** NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′- dichloro-*N*-methyl-, hydrochloride; nitrogen mustard

**Methotrexate:** NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

**Mitotane:** NSC-38721; CAS reg. No. 53-19-0; ethane, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-; *o,p*′-DDD

**Vincristine:** NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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# Cyclophosphamide (NSC-26271) Maintenance Therapy After a Second Remission of Childhood Acute Lymphoblastic Leukemia: Comparative Clinical Trial (Standard Dose Versus Intermittent High Dose Versus Cyclophosphamide Plus Cytosine Arabinoside [NSC-63878])[1]

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## SUMMARY

**Children with acute lymphoblastic leukemia who had experienced only one relapse were reinduced into remission using a 6-week induction course of prednisone and vincristine. One hundred fifty-one children who achieved a second complete marrow remission were randomly assigned to one of three cyclophosphamide treatment groups for maintenance. Forty-one children received standard-dose cyclophosphamide (3 mg/kg/day), 55 received intermittent high-dose cyclophosphamide (10 mg/kg/day for 4 days out of 14), and 55 received a combination of oral cyclophosphamide (3 mg/kg/day) plus cytosine arabinoside (3 mg/kg/week im). The standard-dose cyclophosphamide regimen resulted in a remission maintenance time of 109 days and was the least toxic of the three maintenance regimens. Giving cyclophosphamide on an intermittent high-dose schedule or combining it with cytosine arabinoside did not increase the remission maintenance time (105 days).**

[Cancer Chemother Rep 59:1097-1102, 1975]

In 1958 cyclophosphamide was reported to have antitumor activity (1) and activity against acute childhood leukemia was reported in 1960 (2). The results of the Children's Cancer Study Group (CCSG) published in 1966 (3) showed that standard-dose daily cyclophosphamide given orally produced 27.5% $M_1$ marrow remissions in children with acute leukemia who had been previously treated with chemotherapy but who had never received cyclophosphamide before. The mean remission duration was 112 days. (The latest CCSG criteria for leukemia response appear in an appendix in ref 4.)

The purpose of the present study was to determine if the remission maintenance time resulting from treatment with cyclophosphamide could be extended by giving cyclophosphamide in an intermittent high-dose schedule or by combining daily oral cyclophosphamide with cytosine arabinoside. The cyclophosphamide trial with an intermittent high-dose schedule was prompted by improved results against childhood solid tumors when cyclophosphamide was given

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on an intermittent high-dose schedule as reported by Finklestein et al (5). It was thought that a similar schedule might also be effective in childhood leukemia.

Cytosine arabinoside was chosen to use in combination with cyclophosphamide in an attempt to improve the maintenance duration on the basis of clinical evidence and animal data, as well as on theoretical considerations. The combination of cytosine arabinoside plus cyclophosphamide appeared to have a synergistic effect when tested in the L1210 animal model. Schabel (6) used cytosine arabinoside plus a nitrosourea compound in the original L1210 study but Bodey et al (7) reported that the same results were obtained when cyclophosphamide was used with cytosine arabinoside instead of a nitrosourea. Furthermore, Bodey reported 70% complete bone-marrow remissions in a small number of children with acute lymphoblastic leukemia (ALL) using a combination of cytosine arabinoside plus cyclophosphamide. This result was superior to the CCSG experience with cyclophosphamide mentioned above and was also superior to the 35% remission rate reported by Howard et al (8) when cytosine arabinoside was used alone. The combination would seem to be of value on a theoretical basis because cyclophosphamide is active against preformed DNA and cytosine arabinoside inhibits DNA synthesis; therefore, combination treatment would constitute a form of concurrent chemotherapy (9).

## MATERIALS AND METHODS

Children with acute lymphoblastic, stem cell, or undifferentiated leukemia who were previously treated with, and resistant to, methotrexate and 6-mercaptopurine were eligible for this study. They were permitted one previous course of steroids but were ineligible if previously treated with vincristine, cyclophosphamide, or cytosine arabinoside. Most of the patients entering this study were expected to come from a single prior group protocol (CCSG No. 803) (10) and remained eligible if they had received one injection of mechlorethamine every 8 weeks as directed by the previous protocol. An $M_3$ bone marrow was a requirement for admission to this study.

Induction treatment consisted of prednisone (2 mg/kg/day for 28 days, tapered to no drug by 42 days) plus vincristine (0.075 mg/kg/week × 6) (fig 1). If the bone marrow was $M_1$ on Day 42, the patient was eligible to continue into the remission maintenance phase. If the marrow was $M_2$ or $M_3$ the child was removed from the study.

Patients achieving an $M_1$ marrow were randomly assigned to one of three treatment programs, and children who had previously received mechlorethamine were randomized separately among the same three groups. The three treatment groups were as follows: group I—cyclophosphamide (3 mg/kg/day orally); group II—cyclophosphamide (10 mg/kg/day × 4 days orally beginning on Day 0 of maintenance and given every 14 days thereafter); and group III—cyclophosphamide (3 mg/kg/day orally) plus cytosine arabinoside (3 mg/kg/week im) with both drugs beginning on Day 0 of the remission maintenance program.

Every 28 days during maintenance the observations required were physical examination, complete blood cell count, and bone-marrow aspiration. Provisions were made in the protocol for dosage modifications in the event of hematologic toxicity. In addition, cyclophosphamide was to be discontinued if hemorrhagic cystitis occurred.

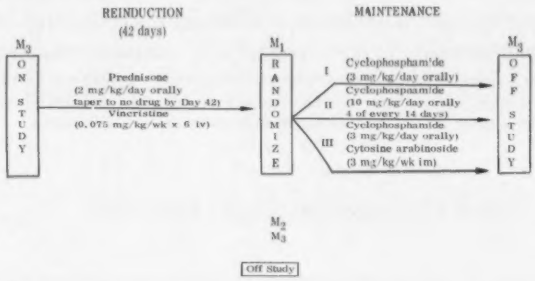


FIGURE 1.—Schematic diagram of reinduction and maintenance protocol.

Central nervous system (CNS) leukemia was to be treated with intrathecal methotrexate and patients were not to be removed from the study if this complication developed.

An $M_3$ marrow during the maintenance phase or uncontrollable toxicity were the indications for removing the patient from the study. Nonparametric methods were used in the analysis of these data and in tests of significance (11).

## RESULTS

Between November 1968 and March 1970, 200 children were admitted to this study. Twelve children were rejected from the induction phase because of failure to follow the protocol. Of the remaining 188 children, four died during induction and 29 failed to achieve an $M_1$ marrow. Thus, 155 children (82.5%) achieved an $M_1$ marrow. Of this group, two children who achieved an $M_1$ marrow were taken off the study during the induction by the investigator because of severe vincristine toxicity, and two children were not randomized into the maintenance groups because of administrative problems. The remaining

TABLE 1.—Time on maintenance therapy: comparison of standard-dose cyclophosphamide, high-dose cyclophosphamide, and cyclophosphamide plus cytosine arabinoside

| Group* | No. of patients | Median time on maintenance in days (range) |
|---|---|---|
| I | 41 | 109 (14-307) |
| II | 55 | 105 (2-687) |
| III | 55 | 105 (23-497) |
| Prior treatment with mechlorethamine | | |
| I | 17 | 105 |
| II | 26 | 100 |
| III | 26 | 78 |
| No prior treatment with mechlorethamine | | |
| I | 23 | 121 |
| II | 28 | 102 |
| III | 29 | 115 |
| Prior mechlorethamine treatment unknown | | |
| I | 1 | |
| II | 1 | |
| III | 0 | |

*Group I = cyclophosphamide (3 mg/kg/day orally); group II = cyclophosphamide (10 mg/kg/day orally × 4 of every 14 days); group III = cyclophosphamide (3 mg/kg/day orally) plus cytosine arabinoside (3 mg/kg/wk im).

151 patients were randomized to three maintenance regimens: group I (41 patients) and groups II and III (55 patients each). There was no significant difference between the three groups with regard to the median durations of remission, which were 109, 105, and 105 days respectively (table 1).

As mentioned above, randomization into the three groups was done separately for those children who had received mechlorethamine as part of the previous study (table 1). The difference in remission duration is not significant ($P = 0.69$). This separate randomization for children who had received mechlorethamine previously was necessary because both cyclophosphamide and mechlorethamine are alkylating agents and resistance to cyclophosphamide might have developed because of the previous exposure to mechlorethamine.

Previous treatment with pulse doses of mechlorethamine was the only factor taken into consideration at the time of randomization, but other factors which might have prognostic significance were also examined. These included white blood cell count at the time of diagnosis, age at diagnosis, length of previous treatment, and CNS leukemia before entering this study. No significant differences were observed between the patient groups (table 2).

Eleven children were taken off the study with an $M_1$ marrow during maintenance because of toxicity, failure to follow protocol, extramedullary leukemia, or death, but were included in the final evaluation of the time on maintenance. This analysis was possible because life-table methods were used.

The percent of children who did not show any hematologic, genitourinary (GU), gastrointestinal (GI), or "other" types of toxicity was 39% in group I, 25% in group II, and 16% in group III. Life-threatening toxicity occurred in eight patients as shown in table 3, but it was hematologic toxicity that actually limited the amount of drugs received by the patients.

The median hematologic toxicity values of each group were not significantly different as shown in table 4, but the amount of drug actually taken by the patients was decreased in all groups. Group I patients received a median of 86% of the calculated dose, group II received 72%, and group III received 80% of the cyclophosphamide and 66% of the cytosine arabinoside.

Complications were infrequent during maintenance in all of the groups. Only five instances were recorded. These included one instance of sepsis and four severe infections other than sepsis. These complications were equally divided among the three maintenance groups.

CNS leukemia was a frequent occurrence during this study. None of the children had received prophylactic CNS therapy prior to entry. Of 151 children in maintenance, 50 entered with a prior history of CNS disease. During the course of this study CNS disease developed in 15 of 41 patients in group I, in 18 of 55 patients in group II, and in 12 of 55 patients in group III. The breakdown of CNS leukemia occurrence before and during this study is shown in table 3.

Forty-seven children with CNS leukemia were treated with only intrathecal methotrexate, seven others received intrathecal methotrexate plus cranial radiation, and in five patients cranial-spinal radiation therapy without methotrexate was used. Extramedullary leukemia other than CNS disease occurred in three children.

The addition of methotrexate given intrathecally to 54 patients in this study represents an additional chemotherapeutic agent given to these children and could have prolonged their remission maintenance time. When this possible effect was analyzed, it was found not to be significant ($P = 0.79$).

## DISCUSSION

The combination of cyclophosphamide and cytosine arabinoside or intermittent high-dose cyclophosphamide was not shown to be better for maintenance than daily standard-dose cyclophosphamide.

TABLE 2.—Time on maintenance therapy comparing standard-dose cyclophosphamide, high-dose cyclophosphamide, and cyclophosphamide plus cytosine arabinoside, with analysis of subgroups for additional factors having possible prognostic value

| Subgroups analyzed | Groups* | | | | | |
|---|---|---|---|---|---|---|
| | I | | II | | III | |
| | No. of patients | Median time on maintenance (days) | No. of patients | Median time on maintenance (days) | No. of patients | Median time on maintenance (days) |
| Wbc | | | | | | |
| < 20,000 cells/mm³ | 25 | 128.0 | 35 | 112.0 | 33† | 98.0 |
| > 20,000 cells/mm³ | 16 | 99.0 | 20 | 61.5 | 20 | 98.0 |
| Age | | | | | | |
| < 3 yrs | 16 | 99.0 | 12 | 132.5 | 13 | 115.0 |
| > 3 yrs | 25 | 119.0 | 43 | 85.0 | 42 | 88.0 |
| Prior therapy | | | | | | |
| < 2 yrs | 37 | 119.0 | 50 | 96.5 | 50 | 93.0 |
| > 2 yrs | 4 | 84.0 | 5 | 149.0 | 5 | 112.0 |
| No CNS leukemia this study | | | | | | |
| With previous CNS leukemia | 8 | 144 | 5 | 60 | 4 | 84 |
| Without previous CNS leukemia | 22 | 112 | 27 | 108 | 26 | 105 |
| CNS leukemia this study | | | | | | |
| With previous CNS leukemia | 7 | 114 | 14 | 157 | 13 | 144 |
| Without previous CNS leukemia | 4 | 144 | 9 | 126 | 12 | 151 |

*See table 1.
†Wbc counts were not known for 2 patients in group III.

TABLE 3.—No. of patients showing life-threatening toxicity*

| Group† | Toxicity | | | |
|---|---|---|---|---|
| | Hematologic | GU | GI | Other‡ |
| I | 0 | 1 | 0 | 0 |
| II | 2 | 0 | 1 | 0 |
| III | 1 | 2 | 0 | 1 |

*Grade 4 toxicity as defined by CCSG toxicity criteria.
†See table 1.
‡The single case was recurrent jaundice associated with cytosine arabinoside administration.

TABLE 4.—Medians and ranges for the hematologic toxicity parameters analyzed

| Parameter | Groups* | | | | | |
|---|---|---|---|---|---|---|
| | I | | II | | III | |
| | Median | Range | Median | Range | Median | Range |
| Hemoglobin (end of induction) | 11.0 | 7.4-15.3 | 10.8 | 4.4-14.0 | 11.0 | 7.7-15.1 |
| Wbc (end of induction) | 6.6 | 1.8-22.7 | 5.5 | 1.3-23.5 | 6.8 | 1.8-20.5 |
| Platelet count (end of induction) | 222.5 | 22.3-735.0 | 205.0 | 75.0-659.0 | 243.0 | 61.0-904.0 |
| Hemoglobin on day of lowest wbc | 10.8 | 6.7-13.6 | 10.5 | 4.4-14.8 | 10.2 | 7.1-14.2 |
| Wbc nadir | 2.2 | 0.7-7.5 | 1.9 | 0.2-11.1 | 1.7 | 0.4-4.6 |
| Granulocyte % at wbc nadir | 55.0 | 27.0-89.0 | 45.0 | 2.0-82.0 | 51.5 | 1.0-85.0 |
| Nadir platelet count | 168.5 | 84.0-450.0 | 106.0 | 5.0-400.0 | 122.0 | 23.6-260.0 |
| Days to wbc nadir | 39.0 | 1.0-238.0 | 27.5 | 6.0-226.0 | 41.5 | 13.0-345.0 |
| Days to nadir platelet count | 51.5 | 6.0-168.0 | 34.0 | 6.0-226.0 | 31.0 | 7.0-345.0 |
| Days of previous disease | 280.0 | 82.0-1419.0 | 329.0 | 126.0-1339.0 | 339.0 | 45.0-1540.0 |
| (% protocol dose received) | | | | | | |
| Cyclophosphamide | (86.0) | (27.0-119.0) | (72.0) | (25.0-111.0) | (80.0) | (15.0-149.0) |
| Cytosine arabinoside | | | | | (66.0) | (15.0-125.0) |

*See table 1.

All three regimens are productive of a median remission maintenance time in the range of 105-109 days, but the daily standard dose may be superior because of its lesser toxicity.

The median remission duration of 109 days does not recommend cyclophosphamide as a valuable remission maintenance drug, especially when one considers that an unmaintained remission following vincristine induction in children with leukemia who were previously treated with chemotherapy but never with vincristine typically lasts from 55 to 59 days (12,13).

The overall toxicity of cyclophosphamide seemed to be least severe at a dose of 3 mg/kg/day and most severe when cyclophosphamide was used in combination with cytosine arabinoside. This was clearly shown with respect to the extremes of toxicity because 39% of the group I patients were free from toxicity opposed to only 16% of those on the combination therapy in group 3. The medians in each of the toxicity categories of hematologic, GI, GU, or "other" were not significantly different. When these toxicity figures are correlated with the percent of calculated drug dose received by the patients in the three groups, it would clearly seem that the results of the study could not have been improved by giving a higher dose because the children could not have tolerated more drug. Even in the least toxic group there were 61% who showed some toxicity despite the fact that the actual dose of drug that they received was 24% less than the calculated drug dose of 3 mg/kg/day.

CNS leukemia was frequently seen in all three groups and none of the children in any of these groups seemed to be "protected" from CNS leukemia because of the drug dose given or the combination of drugs used. In a previous CCSG study it was suggested that cytosine arabinoside might protect children against the development of CNS leukemia (8).

In conclusion, standard daily-dose cyclophosphamide, intermittent high-dose cyclophosphamide, and cyclophosphamide plus cytosine arabinoside do prolong remission maintenance time beyond that of a no-therapy maintenance program in children with ALL or acute undifferentiated leukemia who have relapsed after previous treatment with drugs other than those used in this study. The remission maintenance qualities, however, are not enhanced by giving cyclophosphamide in an intermittent high-dose schedule or by combining it with weekly injections of cytosine arabinoside as done in this study.

## LIST OF COMPOUNDS

**Cyclophosphamide:** NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

**Cytosine arabinoside:** NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

**Mechlorethamine:** NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,-2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard

**6-Mercaptopurine:** NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

**Methotrexate:** NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

**Prednisone:** NSC-10023; CAS reg. No. 53-03-2; pregna-1, 4-diene-3,-11, 20-trione, 17, 21-dihydroxy-

**Vincristine:** NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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# Urinary Excretion of Polyamines by Patients With Advanced Malignancy[1,2]

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## SUMMARY

Levels of putrescine, spermidine, and spermine in urine were determined by means of a sensitive ion-exchange chromatographic method in patients with advanced solid tumor malignancies, in patients with diseases other than cancer, and in normal control subjects. Elevations above 2 SDs of the normal mean were found in a varying number of patients in each tumor category. For those malignancies studied that involved more than 20 patients, the greatest incidences of increased excretion were 66% for spermine in patients with colon carcinoma and 50% for putrescine and spermidine in patients with bronchogenic carcinoma. The highest levels and greatest frequency of elevated polyamine levels were found in patients with Burkitt's lymphoma, and changes in clinical tumor status associated with treatment appeared to correlate well with polyamine levels in this disease. Abnormal amounts of polyamines were also excreted by some patients with diseases other than cancer, indicating that increased polyamine excretion is not restricted or specific to the neoplastic state. It was also found that the levels of polyamines were apparently not affected by the intake of meat or the diet eaten, and remained in a rather narrow excretion range for any one individual at different time intervals. This study was carried out as part of a program to determine and evaluate biologic materials present in body fluids that may be used to follow and evaluate response or progression of neoplastic disease in patients during treatment regimens. The results suggest that abnormal urinary polyamine levels may be characteristic of neoplastic growth for some patients with malignant disease. Further studies are necessary to determine if these compounds may be helpful in assessing disease status for patients with such solid tumor malignancies as colon and bronchogenic carcinoma although their potential as useful "biologic markers" appears less promising than originally anticipated.

[Cancer Chemother Rep 59:1103-1116, 1975]

Polyamines, including putrescine, spermidine, and spermine, are normal urinary excretion products. Their possible value in the diagnosis of cancer and for monitoring patients during therapy was stimulated by the initial reports of Russell et al (1,2) indicating polyamine levels in urine samples were frequently elevated for patients with malignancy. For the individual patients in these reports, surgical removal of a tumor mass or disease remission after the administration of chemotherapeutic agents was

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accompanied by a decrease in abnormal urine polyamine levels (2), and for patients with diseases other than cancer the amounts excreted were essentially the same as for normal control subjects (2). However, the method of analysis used by these investigators lacked adequate sensitivity to determine the normal control range of values and was not sufficiently specific, particularly in the accurate estimation of spermine (3). Subsequently, procedures utilizing gas-liquid (4,5) or cation-exchange chromatography with an amino acid analyzer (6,7) have been developed which provide the sensitivity, specificity, and accuracy necessary for the analysis of urinary polyamines. Using an automated ion-exchange method (6), we have determined polyamine levels in urine samples from patients with advanced neoplastic disease including a variety of different tumor types and, for comparison, in urine samples from normal control subjects and from patients with diseases other than cancer. To our knowledge, this is the first report of urinary polyamine levels in a large group of patients with advanced solid tumor malignancy, in patients with diseases other than cancer, and in normal subjects.

## MATERIALS AND METHODS

Urine specimens (24-hour), which were refrigerated without preservative during the collection period, were obtained from all patients and normal control subjects. Aliquot samples were frozen immediately and were maintained at -50°C or lower until analysis. The patients with cancer had metastatic disease but were eligible for and were entered into chemotherapy phase II or III studies. The urine was collected prior to antitumor chemotherapy and at a time when no specific cancer therapy was being administered. The diseases other than cancer included a variety of acute and chronic recurrent disorders. In each patient in the study, the disease was in an active phase, with the patient having overt signs and symptoms of the disorder. At the time urine samples were obtained, all of these patients required and were receiving specific, frequently multiple, drug therapy. Urine samples from children (5-16 years old) with Burkitt's lymphoma were collected before chemotherapy and after antitumor response, at a time when the patient was considered to be either disease-free or had had a marked reduction in tumor mass as measured by clinical means; control urine samples were obtained from normal subjects in the same age range.

### Methods of Analysis

The procedure of Gehrke et al (6) for the analysis of polyamines in urine was used to determine quantitatively the amounts of putrescine, spermidine, and spermine in each individual aliquot sample of urine. Because of the resolution and sensitivity of the method, cadaverine could also be assayed by this method, thus, cadaverine was determined for some of the control subjects and for the patients with cancer. The method utilizes a Bio-Cal model BC-200 amino acid analyzer with a 7.5 × 0.9-cm column packed with Aminex A-5 (12-15 $\mu$) spherical resin equipped with a chromatronix ROSV-1.0 20-loop, refrigerated, automatic injection valve. A model MV-3 HP 3-port motor valve, inserted between the resin column and the ninhydrin reaction coil, was controlled by the electronic programmer of the analyzer. The chromatographic peak areas were determined with an Infotronic model 100-A digital integrator with a baseline display connected between the 570-nm colorimetric detector and the Honeywell Electronik 15 multipoint recorder with a 50 mV, 0-2 OD span. Details regarding the instrumentation and its use, the reagents and buffers, the cation-exchange chromatography analysis sequence, the preparation of urine samples for analysis, the standard curves, the precision of the method, and the analytic results for specific urine samples are fully described in a recent publication (6). Average recovery of individual polyamine standards added to urine was 96% for putrescine, 97% for cadaverine, 95% for spermidine, and 97% for spermine (6).

## RESULTS

Figures 1 and 2 illustrate the cation-exchange separation of the diamines and polyamines, with ninhydrin detection and measurement at 570 nm. Experimental and chromatographic details are presented in reference 6. Figure 1 presents the analysis of a standard calibration solution of the amines, and figure 2 is a typical representative chromatogram obtained from a urine sample from a patient with cancer.

Analytic data in table 1 demonstrate the precision achieved using the cation-exchange chromatographic method for assay and are in essential agreement with precision studies previously reported (6). Two aliquot portions each of individual urine samples from 28 subjects were hydrolyzed and analyzed independently on different days and the levels were compared. As demonstrated, close agreement in the results for the individual aliquot samples was obtained for each one of the respective polyamines. It should be noted that the higher average relative standard deviations (RSDs) for cadaverine (14.0%) and for spermine (13.5%) were indicative of the inverse relationship between polyamine levels and average RSDs, ie, the lower the polyamine levels the higher the average RSDs.

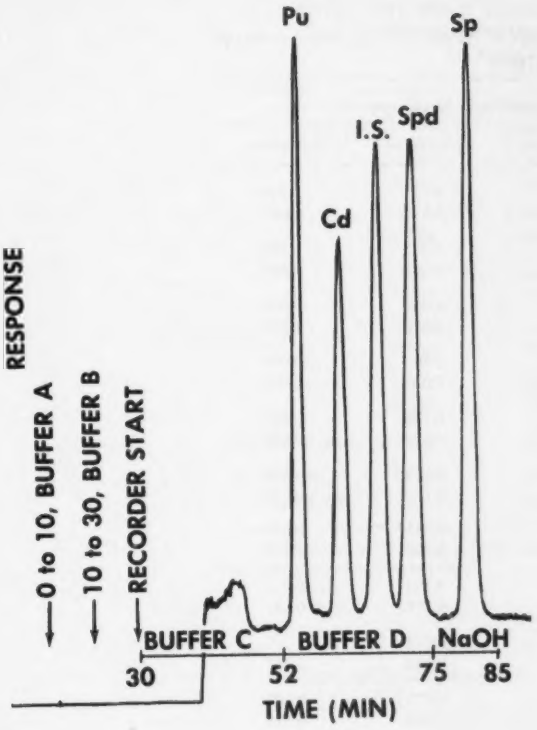


FIGURE 1.—Cation-exchange analysis of standard calibration, mixture of the four diamines and polyamines. Internal standard = 3,3'-diaminodipropylamine. Sample injected = 6 μg of each polyamine.

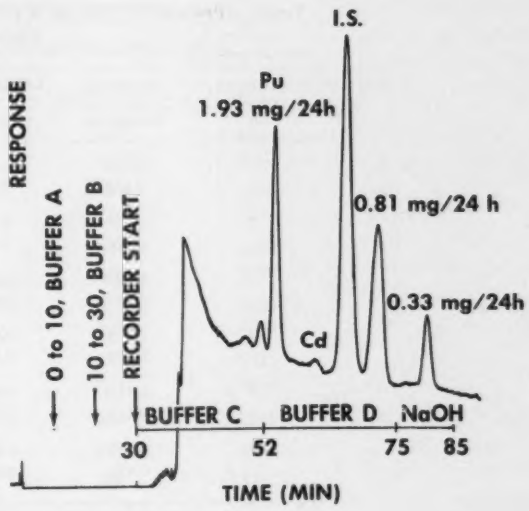


FIGURE 2.—Cation-exchange analysis of a urine sample from a patient with breast cancer. Internal standard = 3,3'-diaminodipropylamine. Sample injected = 1.25 ml of hydrolyzed urine.

The urinary excretion levels for putrescine, spermidine, and spermine in normal control subjects, in patients with diseases other than cancer, and in patients with advanced solid tumor malignancies are shown in figures 3-7. The levels are all expressed in μmol of the polyamine/kg of body weight/24-hour urine collection (μmol/kg/24 hrs).

For normal control subjects, a distinct sex difference was found in the mean level and range for putrescine (figs 3 and 4), and to a lesser extent for spermine (figs 6 and 7). Females without disease excrete greater amounts of putrescine than males, whereas males excrete greater amounts of spermine than females. The mean values for spermidine and the range were essentially the same (fig 5) for both males and females. The differences observed for putrescine and spermine excretion for females did not appear to be related to menstrual status although a decrease in the average level for putrescine was noted with increasing age. This age-related difference was not found in males. We have found that children excrete somewhat greater amounts than adults when based on weight (8).

To provide further data as to the accuracy of the method, the variation in individual polyamine value was determined based on a random series of 30 individual urine specimens from normal control subjects and from patients with cancer and was analyzed in sequence in duplicate. For putrescine the average deviation from the mean of the two determinations per specimen was ± 0.009 μmol; for spermidine and spermine this value was ± 0.001 μmol.

Polyamine levels were determined in two normal subjects at four intervals of several days to weeks in order to evaluate the normal variation in excretion amounts with time. For the female, the range was 0.286 μmol/kg/24 hrs for putrescine, 0.045 μmol for spermidine, and 0.005 μmol for spermine. For the male, the range was 0.127 μmol/kg/24 hrs for putrescine, 0.025 μmol for spermidine, and 0.010 μmol for spermine. To evaluate the possibility that dietary factors might affect the amount of polyamines excreted, five males (normal subjects) were placed on a meat-free (low-purine) diet for 3 days prior to analysis and thereafter ate a regular diet with an added portion of meat at each meal for the next 3 days when a second specimen was collected for polyamine determination. The results of this study are presented in table 2. As shown, the levels of polyamines were apparently not affected by the addition of meat or by the diet consumed and were within the normal range on both occasions, again demonstrating a rather narrow range of excretion values for any one individual at two different time intervals.

TABLE 1.—Precision of analysis of polyamines in human urine by cation-exchange chromatography*

| Sample No. | Levels in μmol/kg/24-hr collection of— | | | |
|---|---|---|---|---|
| | Putrescine | Cadaverine | Spermidine | Spermine |
| 1 | 0.506 | 0.014 | 0.155 | 0.011 |
| | 0.488 | 0.015 | 0.155 | 0.011 |
| 2 | 0.156 | 0.037 | 0.043 | 0.007 |
| | 0.156 | 0.034 | 0.044 | 0.007 |
| 3 | 0.332 | 0.052 | 0.071 | 0.007 |
| | 0.336 | 0.047 | 0.072 | 0.005 |
| 4 | 0.402 | 0.009 | 0.093 | 0.007 |
| | 0.420 | 0.009 | 0.093 | 0.007 |
| 5 | 0.410 | 0.018 | 0.129 | 0.016 |
| | 0.410 | 0.018 | 0.129 | 0.016 |
| 6 | 0.447 | 0.034 | 0.129 | 0.018 |
| | 0.431 | 0.036 | 0.127 | 0.019 |
| 7 | 0.377 | T† | 0.084 | 0.009 |
| | 0.378 | T | 0.085 | 0.006 |
| 8 | 0.567 | T | 0.184 | 0.026 |
| | 0.470 | T | 0.170 | 0.031 |
| 9 | 0.405 | 0.034 | 0.079 | 0.010 |
| | 0.388 | 0.039 | 0.084 | 0.007 |
| 10 | 0.289 | 0.015 | 0.075 | 0.009 |
| | 0.254 | 0.012 | 0.070 | 0.010 |
| 11 | 0.376 | 0.015 | 0.084 | 0.017 |
| | 0.364 | 0.018 | 0.084 | 0.017 |
| 12 | 0.579 | 0.008 | 0.134 | 0.017 |
| | 0.554 | 0.010 | 0.138 | 0.021 |
| 13 | 0.242 | 0.003 | 0.075 | 0.008 |
| | 0.220 | 0.004 | 0.076 | 0.010 |
| 14 | 0.372 | 0.105 | 0.076 | 0.007 |
| | 0.367 | 0.090 | 0.073 | 0.008 |
| 15 | 0.354 | 0.027 | 0.083 | 0.012 |
| | 0.383 | 0.028 | 0.077 | 0.008 |
| 16 | 0.396 | 0.007 | 0.051 | 0.009 |
| | 0.392 | 0.014 | 0.051 | 0.012 |
| 17 | 0.271 | 0.016 | 0.101 | 0.012 |
| | 0.269 | 0.018 | 0.102 | 0.016 |
| 18 | 0.268 | 0.017 | 0.129 | 0.007 |
| | 0.258 | 0.016 | 0.132 | 0.004 |
| 19 | 0.377 | 0.005 | 0.116 | 0.017 |
| | 0.380 | 0.005 | 0.113 | 0.017 |
| 20 | 0.591 | 0.021 | 0.194 | 0.021 |
| | 0.574 | 0.017 | 0.181 | 0.021 |
| 21 | 0.288 | 0.015 | 0.074 | 0.016 |
| | 0.317 | 0.009 | 0.075 | 0.016 |
| 22 | 0.593 | 0.007 | 0.173 | 0.014 |
| | 0.616 | 0.006 | 0.175 | 0.016 |
| 23 | 0.342 | T | 0.131 | 0.015 |
| | 0.323 | T | 0.135 | 0.017 |

TABLE 1.—Precision of analysis of polyamines in human urine by cation-exchange chromatography*—Continued

| Sample No. | Levels in μmol/kg/24-hr collection of— | | | |
|---|---|---|---|---|
| | Putrescine | Cadaverine | Spermidine | Spermine |
| 24 | 0.240 | 0.029 | 0.062 | 0.011 |
| | 0.243 | 0.023 | 0.063 | 0.011 |
| 25 | 0.405 | 0.021 | 0.088 | 0.014 |
| | 0.390 | 0.022 | 0.087 | 0.011 |
| 26 | 0.246 | 0.009 | 0.045 | 0.007 |
| | 0.245 | 0.007 | 0.045 | 0.006 |
| 27 | 0.549 | 0.019 | 0.131 | 0.025 |
| | 0.532 | 0.016 | 0.135 | 0.030 |
| 28 | 0.484 | 0.028 | 0.118 | 0.015 |
| | 0.493 | 0.025 | 0.118 | 0.019 |
| Arithmetic mean | 0.384 | 0.022 | 0.104 | 0.013 |
| Average SD | 0.017 | 0.003 | 0.003 | 0.002 |
| Average % RSD‡ | 4.5 | 14.0 | 3.0 | 13.5 |

*Two aliquot portions of individual urine samples were hydrolyzed and analyzed independently on different days.

† T = trace.

‡

$$\%\text{RSD} = \sqrt{\frac{\Sigma(x_1 - x_2)^2}{2P}} \Big/ \bar{x} \times 100.$$

TABLE 2.—Effect of diet on polyamine urine levels

| Subject | Diet* | Levels in μmol/kg/24-hr collection of— | | |
|---|---|---|---|---|
| | | Putrescine | Spermidine | Spermine |
| A | 1 | 0.133 | 0.051 | 0.003 |
| | 2 | 0.127 | 0.057 | 0.009 |
| B | 1 | 0.248 | 0.096 | 0.011 |
| | 2 | 0.225 | 0.092 | 0.012 |
| C | 1 | 0.274 | 0.172 | 0.015 |
| | 2 | 0.299 | 0.115 | 0.019 |
| D | 1 | 0.180 | 0.066 | 0.011 |
| | 2 | 0.298 | 0.091 | 0.011 |
| E | 1 | 0.281 | 0.134 | 0.026 |
| | 2 | 0.151 | 0.071 | 0.019 |

*1 = No meat for 3 days (low-purine diet); 2 = regular diet with added meat portions for 3 days. Urine was collected for analysis on Days 3 and 6.

In figures 3-7, the results show that patients with diseases other than cancer may excrete greater amounts of polyamines than normal control subjects. It should be emphasized that all of these patients had progressive, symptomatic disease and were invariably receiving multiple-drug therapy at the time of urine collection. The effect of multiple-drug therapy on polyamine excretion is unknown. The diseases included a variety of different syndromes. The results for those diseases studied which involved four or more patients are summarized in table 3. Two of four patients with pneumonia excreted increased amounts of all three polyamines, including the highest levels for putrescine and spermine of all of the noncancer patients in the study. Two of two patients with hyperthyroidism had elevated levels of putrescine and spermine, and one patient had an elevated level of spermidine. In general, the greatest incidence of elevations above 2 SDs of the normal mean was found for putrescine (28 of 104 patients [27%]); the second highest incidence was for spermidine (23 of 104 patients [22%]), and the least was for spermine (14 of 104 patients [13%]).

For those tumor types studied involving more than 20 patients, the analytic results for polyamine excretion are presented in figures 3-7. For other solid tumor malignancies involving fewer patients, the results are presented in summary form in table 4. As demonstrated in figures 3-7 elevations of polyamine levels occurred with variation in the frequency and

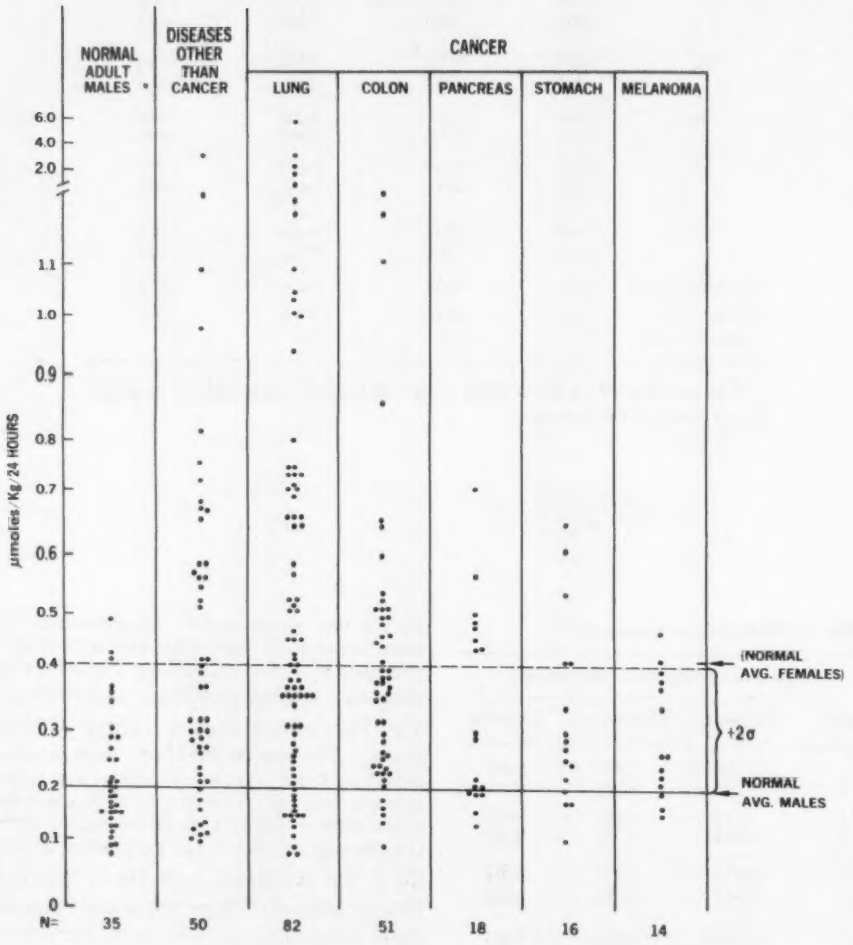


FIGURE 3.—Urinary putrescine levels in normal adult males, in males with diseases other than cancer, and in males with solid tumor malignancies expressed in µmol of putrescine/kg of body wt/24-hr excretion.

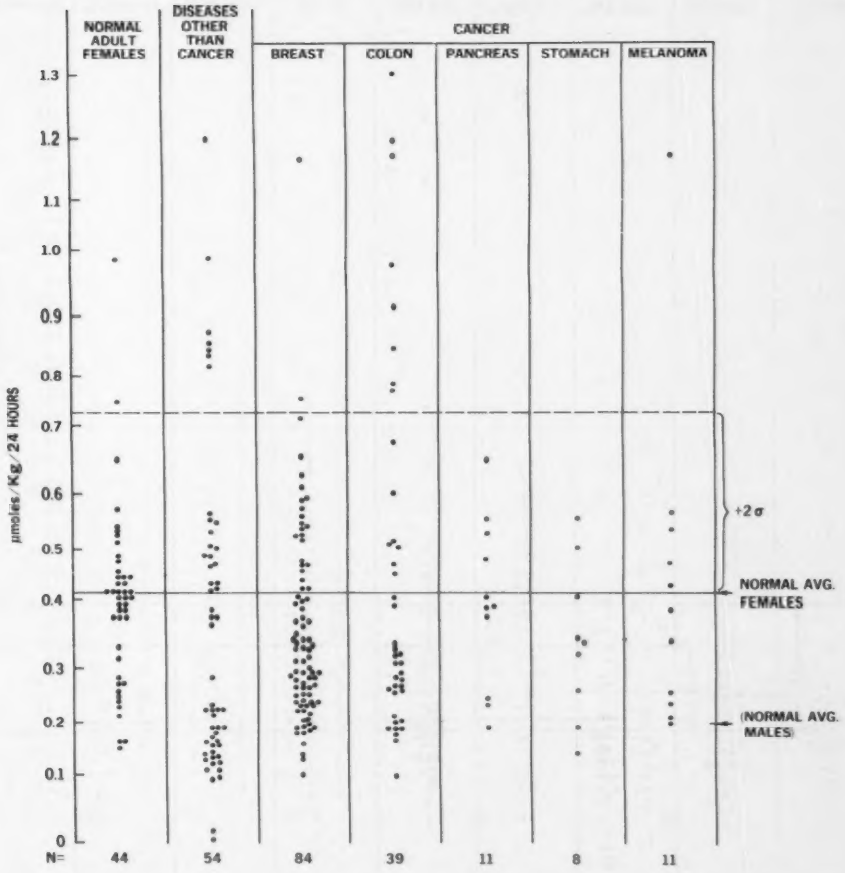


FIGURE 4.—Urinary putrescine levels in normal adult females, in females with diseases other than cancer, and in females with solid tumor malignancies expressed in μmol of putrescine/kg of body wt/24-hr excretion.

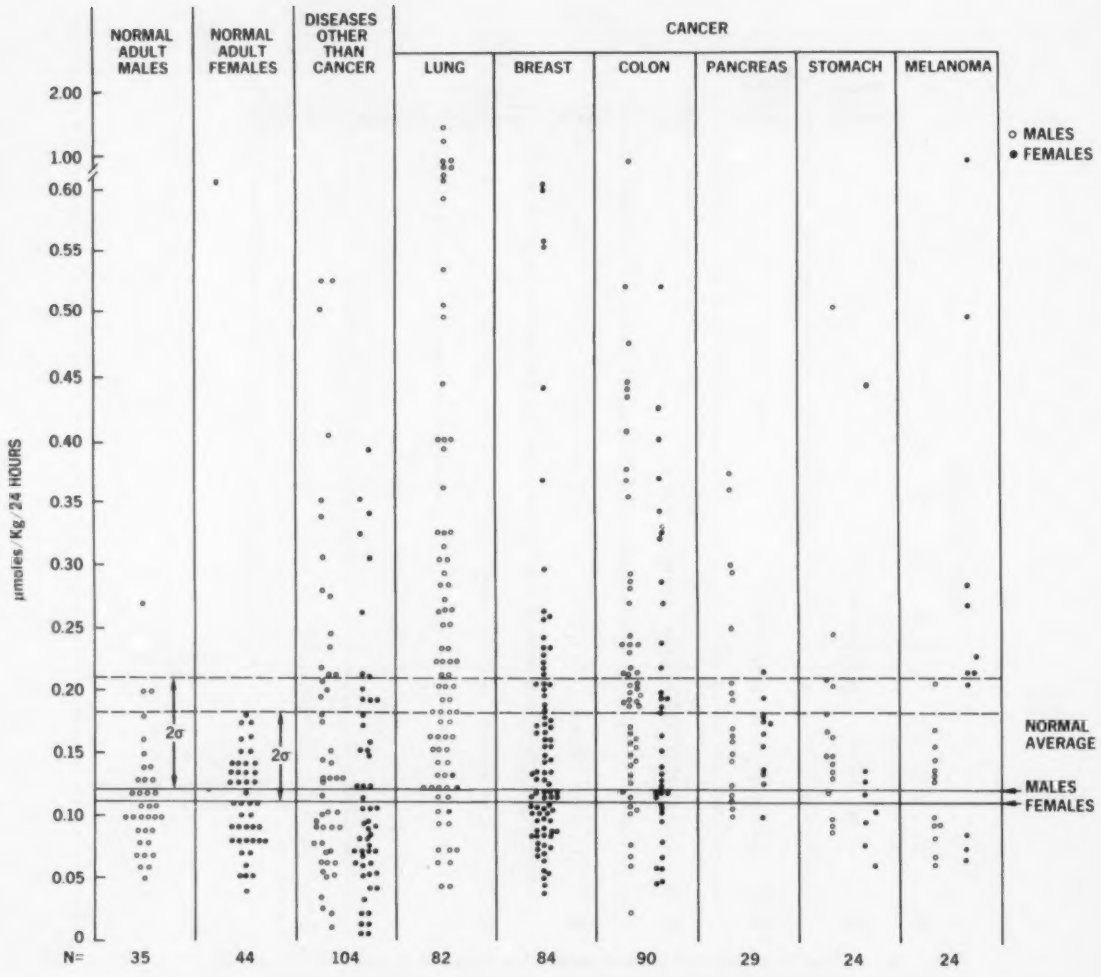


FIGURE 5.—Urinary spermidine levels in normal adult subjects, in patients with diseases other than cancer, and in patients with solid tumor malignancies expressed in μmol of spermidine/kg of body wt/24-hr excretion.

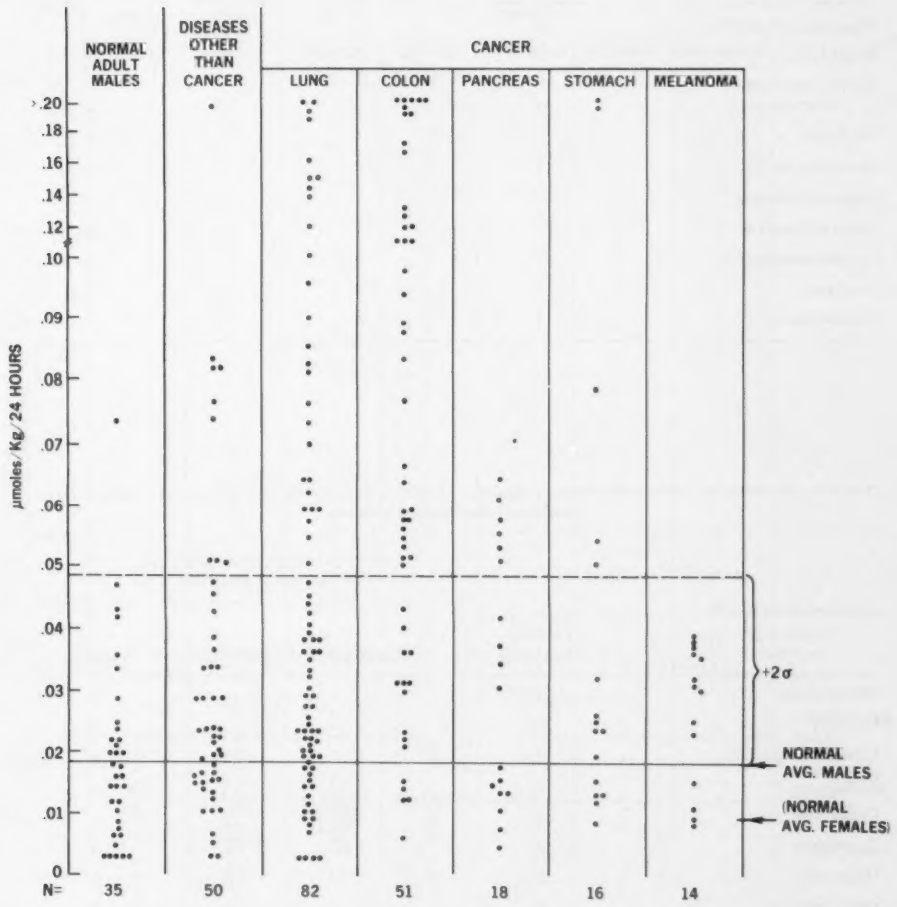


FIGURE 6.—Urinary spermine levels in normal adult males, in males with diseases other than cancer, and in males with solid tumor malignancies expressed as $\mu$mol of spermine/kg of body wt/24-hr excretion.

TABLE 3.—Incidence of increased urinary polyamine excretion in patients with diseases other than cancer which involved four or more patients

| Diseases studied | Total No. of patients | No. of patients excreting > 2 SDs of the normal mean of— | | |
|---|---|---|---|---|
| | | Putrescine | Spermidine | Spermine |
| Rheumatoid arthritis | 14 | 1 | 2 | 3 |
| Renal failure | 8 | 0 | 0 | 0 |
| Chronic obstructive lung disease | 12 | 5 | 2 | 2 |
| Cirrhosis | 6 | 1 | 2 | 0 |
| Ulcerative colitis | 8 | 2 | 0 | 1 |
| Regional enteritis | 7 | 2 | 1 | 1 |
| Acute pancreatitis | 5 | 1 | 2 | 0 |
| Chronic pancreatitis | 10 | 0 | 1 | 1 |
| Psoriasis | 9 | 0 | 1 | 1 |
| Pneumonia | 4 | 2 | 2 | 2 |

TABLE 4.—Incidence of increased urinary polyamine excretion in patients with solid tumor malignancies involving fewer than 20 patients

| Malignancies tested (tumor type or site) | Total No. of patients | No. of patients excreting > 2 SDs of the normal mean of— | | |
|---|---|---|---|---|
| | | Putrescine | Spermidine | Spermine |
| Astrocytoma | 7 | 1 | 1 | 0 |
| Prostate | 4 | 1 | 2 | 1 |
| Osteogenic sarcoma | 4 | 3 | 2 | 3 |
| Kidney | 3 | 2 | 1 | 1 |
| Lymphoma | 3 | 2 | 2 | 3 |
| Esophagus | 3 | 2 | 0 | 0 |
| Hepatoma | 2 | 2 | 2 | 2 |
| Small bowel | 2 | 0 | 0 | 1 |
| Miscellaneous | 12 | 4 | 7 | 4 |

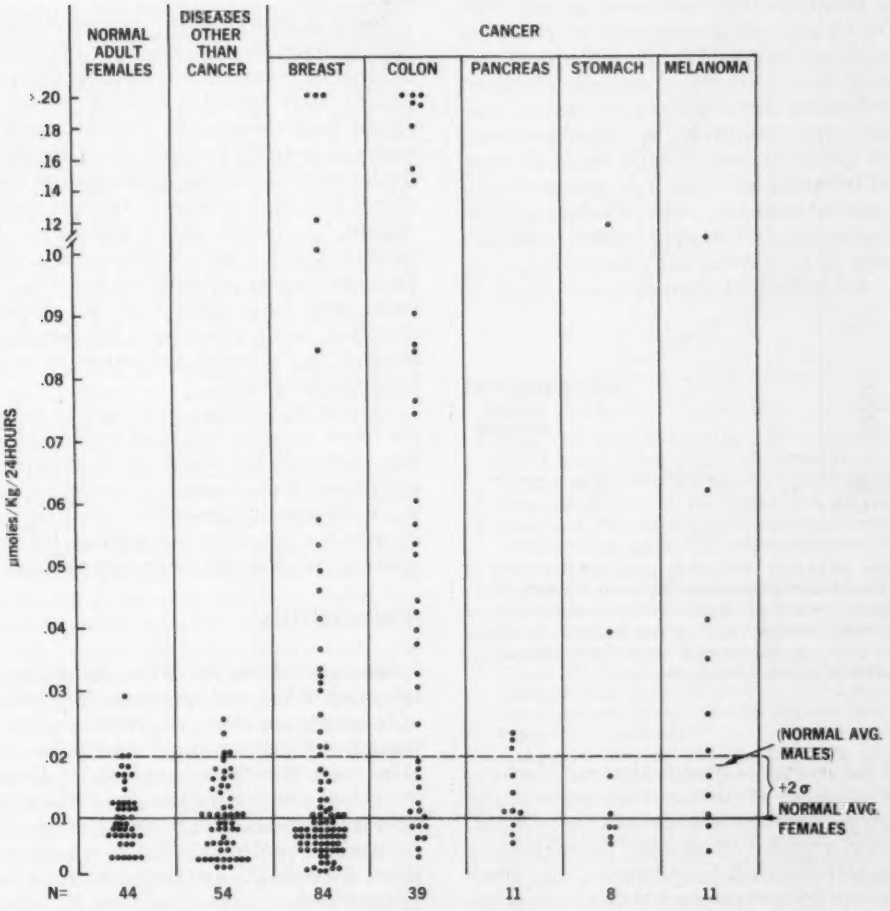


FIGURE 7.—Urinary spermine levels in normal adult females, in females with diseases other than cancer, and in females with solid tumor malignancies expressed in μmol of spermine/kg of body wt/24-hr excretion.

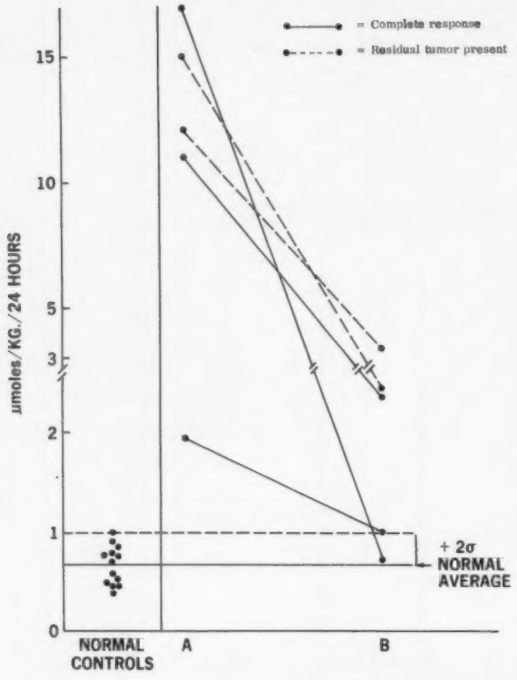


FIGURE 8.—Total polyamine urine levels expressed in μmol/kg of body wt/24-hr excretion in patients (5-16 years old) with Burkitt's lymphoma prior to treatment (*A*) and after one or more courses of drug therapy (*B*). Complete response was measured by clinical means. Total polyamine excretion for normal control subjects in the same age range is given for comparison.

the amount per patient related to the type of malignancy. The incidence of increased excretion of the individual polyamines also appeared to vary depending on the tumor type. For example, approximately 50% of the patients with bronchogenic carcinoma had levels above 2 SDs of the normal mean of a population for putrescine and spermidine, but only 34% had elevated levels of spermine. The incidence of increased levels for patients with colon carcinoma was 31% for putrescine and 39% for spermidine, but was 66% for spermine.

In determining the frequency of polyamine elevation with organ or tissue site of metastasis, a possible correlation was noted for liver involvement. This was particularly true with regard to spermine excretion in patients with colon carcinoma, because of the high incidence of liver metastases only. In patients with the liver as the solitary known site of tumor, 74% had an elevated level for spermine in comparison to the overall incidence of increased spermine excretion of 66%. In those patients without apparent liver metastases, only 25% had an elevated level. In those patients with breast cancer who had liver involvement either alone or in combination with other sites, the frequency of elevated spermine excretion was 38%, whereas the overall incidence was 25%.

Figure 8 shows the urinary excretion values for total polyamines expressed in μmol/kg/24 hrs for children with Burkitt's lymphoma before treatment (A) and again after one or more courses of antineoplastic drug therapy (B). Prompt, rapid, antitumor response occurred for each one of these patients. At the latter time (B), the patients were considered either to be free of disease or residual tumor was still evident by clinical means although substantially reduced in size. These results suggest that polyamine levels appear to correlate well with clinical disease status in patients with this disease. Normal excretion levels are shown for control subjects—children in the same age range as patients with Burkitt's lymphoma.

Cadaverine analyses were carried out on many of the urine samples analyzed for the other polyamines. Although the incidence of elevations was not determined, increases in levels were found for a variety of different malignancies but at a lower frequency than for the other polyamines. On occasion, cadaverine was the only polyamine elevated.

## DISCUSSION

The polyamines, including the diamines, putrescine, spermidine, and spermine, are widely distributed in nature and their concentrations are highest in tissues undergoing rapid metabolic activity (9). Many roles have been proposed for the polyamines in various physiologic processes including transfer ribonucleic acid (tRNA) methylation (10), aminoacylation of tRNA (11), RNA synthesis (12), bacterial cell division (13), and the growth of animal cells in tissue culture (14,15). They have been implicated as possible factors in rapid tissue growth such as regenerating rat liver (16,17) and early embryogenesis of the chick (18) and rat (19). The only known biosynthetic pathway (20) for these polyamines in mammals includes the enzymatic decarboxylation of ornithine to form putrescine followed by the step-wise conversion of putrescine to spermidine by combination with *S*-adenosyl-L-methionine, and of spermidine to spermine by similar reaction. In plants and in some bacteria, putrescine has also been derived from arginine and agmatine (2). Cadaverine, a diamine derived from lysine (2), is not involved in the same biosynthetic pathway as the other polyamines but has been reported present in small quantities in

normal human urine. For a more extensive review of the polyamines, recent publications cover many aspects of polyamine research (3,20-22).

Utilizing an automated ion-exchange chromatographic procedure, Marton et al (23) recently showed that polyamines may be elevated in the cerebral spinal fluid of patients with brain tumors. Nishioka and Romsdahl (24), by a modified liquid chromatographic method, verified the preliminary results of Marton et al (7) which showed that polyamine levels may also be elevated in the blood sera of patients with cancer. Recovery of standards, however, was low with the average being 51.3% for putrescine, 54.7% for spermidine, and 62.0% for spermine. Putrescine was reported to be the most frequently elevated of the three polyamines in the sera of patients with malignant solid tumors. Iversen et al (25), using, with minor modifications, the original method employed by Russell (1), reported that polyamines were elevated in the urine of approximately two thirds of a group of 56 patients with metastatic disease. There was no evidence that the site of metastatic involvement influenced the incidence or amount of polyamines excreted. Elevations were also found in the urine of patients with diseases other than cancer.

On the basis of the results obtained in this investigation, urinary polyamine levels may be increased for some patients in a rather wide variety of advanced solid tumor malignancies suggesting that such increases are a general characteristic associated with neoplastic growth. In addition, elevations occur for patients with disorders other than cancer, indicating that the increased polyamine excretion is not specific to the neoplastic state. The consequent incidence of false-positive values (the substantial number of patients with advanced malignant disease who have normal levels) suggests that the determination of urinary polyamine levels will have little value in the area of cancer diagnosis.

The highest excretion levels we have found to date have been in patients with Burkitt's lymphoma (8). Preliminary studies suggest that polyamine levels may be of value in evaluating the tumor status of patients with this disease during drug therapy, particularly in patients without objective parameters for measuring disease response or progression. Since Burkitt's lymphoma is a rapidly proliferating tumor with a high growth fraction (25), polyamines may also find application in other neoplastic diseases where the rate of cellular multiplication is rapid. The current study dealt primarily with the more slow-growing solid tumors. A similar evaluation is desirable for patients with hematologic malignancies. As suggested by the results in this study, polyamine levels may be helpful in assessing disease status in such solid tumor malignancies as colon and bronchogenic carcinoma. For colon carcinoma, spermine excretion was increased in 66% of the patients. Of possible significance is the fact that only three of the 42 patients (7%) with noncancerous gastrointestinal diseases had an elevation of spermine excretion: one of ten patients with chronic pancreatitis, one of eight with ulcerative colitis, and one of seven with regional enteritis. Further clinical studies are necessary to determine if the amount of spermine excretion will be an aid in evaluating the presence of recurrent disease in postoperative patients with colon cancer or as an additional means for following tumor regression or progression in patients with the more advanced form of the malignancy during antitumor therapy. Although the potential for polyamines as useful biologic markers for patients with malignant disease appears less promising than originally anticipated, the actual role of polyamines in clinical cancer remains to be determined.

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# Guanazole (NSC-1895)—A Phase I Clinical Study[1,2]

**Eulogio M. Caoili, Robert W. Talley, Frank Smith, Philip Salem, and Vainutis K. Vaitkevicius[3,4]**

## SUMMARY

**Two dose schedules of guanazole were used in this phase I clinical study: intermittent prolonged 5-day infusion and intermittent iv bolus twice weekly. Ninety-seven treatment observations were analyzed for toxic effects resulting from the prolonged infusion and 42 from the twice-weekly bolus schedule. The main toxic effect was bone marrow suppression, the frequency and severity of which were intensified by prior chemotherapy or radiotherapy and repetition of guanazole therapy. The leukocyte count was affected more than the platelet count. Partial responses were observed in four patients: two with lung carcinoma, one with prostate carcinoma, and one with melanoma. Further phase II clinical studies of guanazole are indicated.**

[Cancer Chemother Rep 59:1117-1121, 1975]

Guanazole is one of the triazole compounds that has shown significant activity against murine L1210 leukemia but has shown no activity against adenocarcinoma E0771 and Sarcoma 180 (1).[5] The structural formula of guanazole is shown in figure 1. Guanazole is soluble in water in excess of 200 mg/ml and is stable in solution for at least 2 weeks at room temperature (2).[5] Brockman et al (3) found that guanazole preferentially inhibits deoxyribonucleic acid synthesis by inhibition of ribonucleotide reductase and that the effectiveness of the drug is schedule-dependent. In the treatment of L1210 leukemia, the greatest activity was produced when 500 mg/kg

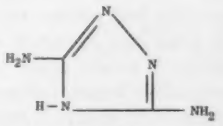

FIGURE 1.—Structure of guanazole.

was given every 3 hours for eight doses and repeated three times with 4-day intervals between courses.[5] Treatment schedules involving once-daily administration were less effective. Gerber et al (4) demonstrated that the drug appeared unchanged in the urine with 95% recovery of the drug in 24 hours. In rat liver perfused with guanazole, no metabolites were detected in the perfusate and approximately 1%-2% appeared as unchanged guanazole in the bile (4).

Animal studies[5] have shown the main toxic effect to be reversible myelosuppression; convulsions, lethargy, prostration, and dyspnea also were observed. When guanazole was administered by rapid injection, peripheral vasodilation, increased central and peripheral venous pressures, and sustained elevation of right ventricular pressure developed. In mice, the toxic dose low of daily iv administration was 8.4-9.6 g/m$^2$. Some activity against human leukemia was recently demonstrated (5).

## MATERIALS AND METHODS

Guanazole was supplied as a dry powder and was stored at room temperature. Each vial contained 5 g and was reconstituted via dilution with 47 ml of dextrose solution or normal saline. Two dose schedules were used: continuous infusion for 120 hours and

[1]Received May 15, 1975; revised Aug 12, 1975; accepted Aug 18, 1975.

[2]Guanazole: CAS reg. No. 503-88-8; 1,2,4-triazolidine, 3,5-diimino-.

[3]This study was conducted by the Southwest Oncology Group (SWOG) and was supported by Public Health Service grants (CA) from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare. The following institutions participated in the study: Wayne State University, Detroit, Mich (*E. M. Caoili, V. K. Vaitkevicius;* (CA-14028, CA-07177); Henry Ford Hospital, Detroit, Mich(*R. W. Talley*; CA-04915); Baylor College of Medicine, Houston, Tex (*Frank Smith*; CA-03392); University of Texas M. D. Anderson Hospital and Tumor Institute, Houston (*Philip Salem*; CA-10379, CA-03754); Cleveland Clinic, Ohio (CA-04919); Wilford Hall USAF Medical Center, Lackland Air Force Base, Tex; Northwestern University Medical School, Chicago, Ill (CA-13236); Scott and White Clinic, Temple, Tex (CA-10187); Borgess Medical Center, Kalamazoo, Mich; Brooke General Hospital, Fort Sam Houston, Tex. Operations Office, SWOG, Kansas City, Kan (CA-12644); and Statistical Office, SWOG, Houston, Tex (CA-12014).

[4]*Reprint requests to:* V. K. Vaitkevicius, MD, Wayne State University School of Medicine, Darling Memorial Center, 4160 John R St, Detroit, Mich 48201.

[5]Livingston RB, and Carter SK. Clinical brochure: guanazole (NSC-1895), Nov 1969. Prepared by the NCI, Bethesda, Md.

twice-weekly iv bolus. Using the conversion factors suggested by Freireich et al (6), the starting dose in human trials should be equivalent to 3.30 g/m$^2$ (one third of the minimal toxic dose in mice). Since a 120-hour infusion was proposed for the study, a lower dose (1.5 g/m$^2$) than the calculated starting dose was utilized. Therapy was repeated every 3 weeks and continued until hematologic toxicity or progression of the disease was observed. The appropriate dose was added to 1000 ml of 5% dextrose in water. In patients in whom no toxicity developed and whose disease was progressing rapidly, a 25% dose escalation was permitted in subsequent courses. Patients with slowly progressive or stationary disease were continued at the same dose level. In patients with toxic effects, the subsequent courses were reduced by 25% of the toxic dose. The intermittent iv bolus dose ranged from an initial dose of 3.5 g/m$^2$ and was gradually escalated in increments of 25% of the previous dose to 30.0 g/m$^2$.

All patients had advanced, histologically proven, malignant disease. All had normal white blood cell (wbc) counts, and none had received any cytotoxic chemotherapy within 3 weeks prior to this study. Patients with congestive heart failure, overt liver failure, or fever caused by active infection were not included. All patients were required to sign an informed consent form. The following laboratory studies were obtained prior to initiation of therapy: complete blood cell count, platelet and reticulocyte counts, urinalysis, creatinine, alkaline phosphatase, serum glutamic oxaloacetic transaminase, prothrombin time, appropriate roentgenograms and measurements of tumor lesions, and, when feasible, bone marrow examination.

For stratification, patients were assigned to either good- or poor-risk groups. Poor-risk patients were defined as follows: those over 65 years old or those with significantly compromised renal or hepatic functions, history of excessive sensitivity to previous myelosuppressive antitumor agents, extensive radiotherapy to bone marrow, or bone marrow infiltration severe enough to result in the presence of circulating nucleated red blood cells.

During the course of treatment, wbc and platelet counts were repeated two or three times a week, renal and hepatic functions were examined weekly, and a complete clinical evaluation was repeated prior to each course of treatment.

Hematopoietic toxic effects were graded as follows:

*Leukopenia*: 0 = none (> 4000 wbc/mm$^3$); 1 = mild (3100-4000 wbc/mm$^3$); 2 = moderate (2100-3000 wbc/mm$^3$); 3 = severe (1100-2000 wbc/mm$^3$); 4 = life-threatening (≤ 1000 wbc/mm$^3$).

*Thrombocytopenia*: 0 = none (> 100,000 platelets/mm$^3$); 1 = mild (76,000-100,000 platelets/mm$^3$); 2 = moderate (51,000-75,000 platelets/mm$^3$); 3 = severe (26,000-50,000 platelets/mm$^3$); 4 = life-threatening (≤ 25,000 platelets/mm$^3$).

Objective tumor response was defined as shrinkage of measurable tumor by at least 50% of the product of its greatest diameters, with no lesions showing any increase in size and no appearance of new metastases.

## RESULTS

### Infusion Study

Sixty-nine patients were entered into this study. Of the 69, five patients died of progressive disease before toxic effects could be evaluated; two were lost to followup. The remaining 62 patients were evaluated for toxicity in a total of 97 treatment observations.

Patients who showed objective response, no evidence of rapid tumor progression, and/or no toxic effects were given additional courses. Twenty-two patients received only one course, 22 received two courses, and 18 received three or more courses. The toxic effects seen in this group of patients are shown in table 1.

Myelosuppression is the major adverse effect of guanazole (table 1). Fifty courses (51%) produced significant leukopenia and 21 courses (20%) produced thrombocytopenia in varying degrees of severity. All patients in whom thrombocytopenia developed had leukopenia. The frequency and severity of myelosuppression were dose-dependent. The lowest dose to produce myelosuppression was 4.5 g/m$^2$. No toxicity developed in one of the three patients receiving 20 g/m$^2$. Megaloblastic changes were seen in all patients who had hematologic toxic effects and whose bone marrows were examined.

With repeated courses, hematologic toxicity increased. Table 2 illustrates this cumulative effect of guanazole. Toxicity developed in six of 20 patients who received a second course of the drug without escalation. Nine patients who showed no hematologic toxic effects after two courses were given a third course without escalation; toxicity developed in five of them. With dose escalation, 92% (22 of 24) of the patients had toxic effects.

In table 3, the hematologic toxic effects of patients with no prior cytotoxic therapy were compared with patients who had received prior myelosuppressive agents, radiation treatment, or chemotherapy administered in combination or sequentially with cobalt 60 radiation. Of the patients who had received prior treatment, 61% showed evidence of myelosuppression, but only 20% showed evidence of myelosuppression in the nontreated group.

TABLE 1.—Toxic effects of guanazole in the 5-day infusion schedule

| Dose ($g/m^2$) | No. of courses | No. of courses with— Leukopenia Grade* 0 | 1 | 2 | 3 | 4 | % | Thrombocytopenia Grade* 0 | 1 | 2 | 3 | 4 | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.50 | 3 | 3 | — | — | — | — | — | 3 | — | — | — | — | — |
| 2.25 | 4 | 4 | — | — | — | — | — | 4 | — | — | — | — | — |
| 3.00 | 8 | 7 | — | 1 | — | — | — | 8 | — | — | — | — | — |
| 3.37 | 4 | 4 | — | — | — | — | — | 4 | — | — | — | — | — |
| 4.00 | 1 | 1 | — | — | — | — | — | 1 | — | — | — | — | — |
| 4.50 | 4 | 3 | — | — | — | 1 | 25 | 3 | — | — | — | 1 | 25 |
| 5.00 | 17 | 10 | 1 | 1 | 4 | 1 | 41 | 15 | — | — | 1 | 1 | 11 |
| 6.00 | 6 | 3 | — | 2 | 1 | — | 50 | 5 | 1 | — | — | — | 16 |
| 7.50 | 29 | 8 | 3 | 6 | 7 | 5 | 72 | 23 | 2 | 3 | — | 1 | 20 |
| 10.00 | 10 | 2 | — | 3 | 4 | 1 | 80 | 7 | — | 3 | — | — | 30 |
| 12.50 | 1 | 1 | — | 1 | 2 | — | 75 | 2 | — | 2 | — | — | 50 |
| 15.00 | 3 | 0 | — | 1 | 1 | 1 | 100 | 0 | — | 1 | 1 | 1 | 100 |
| 17.50 | 1 | 0 | 1 | — | — | — | 100 | 1 | — | — | — | — | — |
| 20.00 | 3 | 1 | — | — | 1 | 1 | 66 | 1 | 1 | — | — | 1 | 66 |
| Total | 97 | 47 | 5 | 15 | 20 | 10 | 51 | 77 | 4 | 9 | 2 | 5 | 20 |

*See Materials and Methods for definitions.

TABLE 2.—Toxicity relationship of repeated treatments with guanazole

| No. of courses | No. of patients receiving repeated treatments with— Same dose Nontoxic | Same dose Toxic | Dose escalation Nontoxic | Dose escalation Toxic |
|---|---|---|---|---|
| 2 | 14 | 6 | 0 | 9 |
| ≥ 3 | 4 | 5 | 2 | 13 |
| Total | 18 | 11 | 2 | 22 |

TABLE 3.—Toxic effect of guanazole in relation to prior treatment

| Prior treatment | No. of courses of guanazole | No. of courses with myelosuppression Grades of severity* 1 | 2 | 3 | 4 | Total | % |
|---|---|---|---|---|---|---|---|
| No treatment | 15 | | | 2 | 1 | 3 | 20 |
| Chemotherapy | 44 | 4 | 7 | 14 | 0 | 25 | 57 |
| Irradiation | 13 | 0 | 3 | 4 | 1 | 8 | 62 |
| Chemotherapy and irradiation | 25 | 1 | 5 | 5 | 3 | 14 | 56 |
| Total | 97 | 5 | 15 | 25 | 5 | 50 | 52 |

*See Materials and Methods for definitions.

### Twice-Weekly Iv Bolus Study

Table 4 summarizes the toxicity produced by guanazole when administered via intermittent iv bolus injections. A total of 56 patients were entered in this study: one was lost to followup, 12 died early in the study, and one refused further treatment after receiving only two doses. Therefore, 42 patients were evaluable for toxic effects.

Leukopenia was the major toxic effect: the median day on which the nadir was reached was Day 17. Recovery from leukocyte depression was uniform with a median duration of 9 days. As shown in table 4, leukopenia occurred in 36% of the patients and thrombocytopenia occurred in 17% of the patients. Leukopenia was observed at all dose levels but with increasing frequency at 15 g/m$^2$ and above. Six of the eight patients in whom leukopenia developed had received prior radiation therapy and/or systemic chemotherapy. Two patients had received neither radiation therapy nor systemic chemotherapy, but leukopenia developed after dose escalation.

The nadir and duration of thrombocytopenia followed the pattern of leukopenia: the median day on which the nadir was reached was Day 14 and the platelet depression usually lasted 6 days. Thrombocytopenia, however, was not as pronounced as leukopenia. In six of the seven patients, thrombocytopenia occurred at a dose level of 10 g/m$^2$ or lower. All six patients had received prior therapy with myelosuppressive agents and/or cobalt 60 radiation.

### Other Toxic Effects

Nonhematologic toxic effects occurred either early (during drug administration) or late (after the drug was administered) during therapy. Early side effects were nausea and vomiting (31 patients), chills and fever (11 patients), somnolence and confusion (eight patients), congestive heart failure (two patients), and cardiac arrhythmia (one patient). While cardiac toxicity was encountered only in the infusion group, other early toxic effects occurred with equal frequency in both the infusion and bolus groups.

Nausea and vomiting occurred early on the 1st day and sometimes persisted for a few days after completion of the 5-day infusion. As a rule, these effects were not serious and could be managed easily with standard antiemetics.

Chills and fever ranging from 38°C to 40°C were seen in patients receiving higher doses. Marked ST-T wave changes developed on one patient's ECG during infusion; two of three patients treated with 20 g/m$^2$ had acute congestive heart failure which failed to respond to digitalization and massive diuretics. All three patients in whom cardiac abnormalities developed had histories of cardiac disease. The large amount of solution infused into these patients may have added to their cardiac symptoms.

Late toxic effects were stomatitis (three patients), dermatitis (one patient), and diarrhea (one patient). No renal or hepatic toxicity was observed.

TABLE 4.—Hematopoietic toxic effects of guanazole in the twice-weekly iv bolus schedule

| Dose (g/m$^2$) | No. of patients treated | No. of patients with— Leukopenia Grade* 1 | 2 | 3 | 4 | % | Thrombocytopenia Grade* 1 | 2 | 3 | 4 | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3.5 | 7 | 1 | 2 | — | — | 43 | — | 1 | — | — | 14 |
| 5.0 | 8 | — | — | 1 | | 13 | — | — | 1 | 1 | 25 |
| 7.5 | 10 | — | — | 1 | — | 10 | — | — | — | 2 | 20 |
| 10.0 | 7 | 2 | 1 | — | — | 43 | — | — | — | 1 | 14 |
| 15.0 | 3 | 2 | — | — | — | 67 | — | — | — | — | — |
| 17.5 | 1 | — | 1 | — | — | 100 | — | — | — | — | — |
| 20.0 | 4 | — | 1 | 2 | — | 75 | 1 | — | — | — | 25 |
| 25.0 | 2 | 1 | — | — | — | 50 | — | — | — | — | — |
| Total | 42 | 6 | 5 | 3 | 3 | 36 | 1 | 1 | 1 | 4 | 17 |

*See Materials and Methods for definitions.

## Antitumor Effect

Partial tumor responses were observed in two patients, one with melanoma and one with prostatic cancer, treated via guanazole infusion. In the twice-weekly study, two patients with carcinoma of the lung had partial tumor responses. One patient with undifferentiated large cell carcinoma of the lung responded for 17 months. Tumor responses were noted both in patients who received low doses (5.0 g/m$^2$) with no hematologic toxicity and in patients who received toxic doses (20 g/m$^2$ twice weekly). Inasmuch as the primary purpose of this study was the evaluation of toxicity, not all patients were evaluable for tumor response. The disease sites in patients who had measurable tumors and who received 5 g/m$^2$ or more of guanazole/day are listed in table 5.

TABLE 5.—Sites of disease in patients who received ≥ 5 g/m$^2$/day and who were evaluable for tumor response

| Type or site of disease | No. of patients treated | No. of partial responses |
|---|---|---|
| Bronchus | 12 | 2 |
| Melanoma | 9 | 1 |
| Kidney | 6 | — |
| Breast | 5 | — |
| Colon | 5 | — |
| Ovary | 2 | — |
| Prostate | 2 | 1 |
| Adenocarcinoma, stomach | 1 | — |
| Adenocarcinoma, unknown primary | 1 | — |
| Appendix | 1 | — |
| Chordoma | 1 | — |
| Fibrosarcoma | 1 | — |
| Liposarcoma | 1 | — |
| Esophagus | 1 | — |
| Osteogenic sarcoma | 1 | — |
| Pancreas | 1 | — |
| Reticulum cell sarcoma | 1 | — |
| Squamous cell carcinoma, external acoustic meatus | 1 | — |

## DISCUSSION

In this clinical study of guanazole, the major toxic effect was myelosuppression. The occurrence of acute congestive heart failure in two patients with compromised cardiovascular status (during slow infusion) requires further study. The mechanism of cardiac toxicity is unknown; however, it has been reported that a rapid infusion of guanazole at high doses increases the central venous pressure and also produces sustained elevation of right ventricular pressure in experimental animals.[6]

Although significant antitumor activity has been demonstrated in the L1210 test system, the therapeutic value was not impressive in this phase I clinical study. Partial responses were observed in two patients with carcinoma of the lung, one patient with melanoma, and one patient with prostatic carcinoma. The duration of response was brief except in one patient with undifferentiated large cell carcinoma of the lung whose response lasted for 17 months. We believe that phase II studies are indicated, especially in patients with melanoma and cancer of the lung and prostate. The recommended doses for further trials are 15 g/m$^2$ in the intermittent 5-day continuous infusion and 20 g/m$^2$ in the intermittent twice-weekly iv bolus. At this time it is not apparent which of the two schedules should be preferred.

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[6] *See* footnote 5.

# Phase I Study of 5-Azacytidine (NSC-102816) Using 24-Hour Continuous Infusion for 5 Days[1,2,3]

**Pavel L. Lomen,[4,5] Laurence H. Baker,[4] Gary L. Neil,[6] and Michael K. Samson [4,7]**

## SUMMARY

**The biologic and antitumor activity of 5-azacytidine has been well demonstrated in the past. The drug at present is thought to be primarily cell cycle phase specific. This study was designed to eliminate undesirable side effects (mainly nausea and vomiting) occurring with a bolus dose and to confirm the recent findings of the relative stability of 5-azacytidine's solution with preserved biologic and antitumor activity. In the study we determined that a dose of 150 mg/m$^2$/day given as a 120-hour continuous iv infusion and repeated at 28-day intervals produced safe, manageable, and reproducible toxicity. The drug was freshly prepared at 4-hour intervals. Eleven courses were administered to seven patients at this dose level and no patient experienced nausea or vomiting. Leukopenia was the major toxic effect. Antitumor activity was shown in one patient with colon cancer and another with American Burkitt's lymphoma.**

[Cancer Chemother Rep 59:1123-1126, 1975]

Within the past 10 years of availability, 5-azacytidine has been widely investigated on a clinical as well as on a theoretical pharmacologic basis (1-7).[8] During clinical trials in the US, it has been established that 5-azacytidine has relatively limited use in solid tumors. However, promising results in the treatment of acute leukemias have been demonstrated (8,9). Besides the low remission rate in solid tumors, the major reason for reluctance in using this drug was severe toxicity—nausea and vomiting as well as bone marrow depression.

In all prior trials, the drug was administered iv or sc daily as a single bolus dose (10,11) for several (3-15) days. The major reason for selecting this schedule was the chemical instability of the drug in solution. Recently, however, some studies[9,10] have shown that there are discrepancies between the biologic activity and the chemical stability data. The compound exhibited almost 90% biologic activity (microbiologic assay) even 24 hours after being dissolved. Data from different laboratories[9,11] also have shown that a stability of the drug in solution is dependent on the pH and concentration.

From the presently available pharmacologic and clinical data (12-15) it is obvious that 5-azacytidine has an extremely complicated pattern of metabolism and degradation in vivo and many questions remain to be resolved. Most reports describe 5-azacytidine as being cell cycle phase specific. There are, however, some recent suggestions that the drug has an additional mode of action and that, besides affecting nucleotide metabolism, it acts by impairing cell structures and therefore can attack resting cells (14).

The data mentioned above served as a basis for conducting a phase I study using a 24-hour continu-

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[2]Supported in part by Public Health Service grants CA-07177 and CA-08096 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]5-Azacytidine: CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-.

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[6]Cancer Research Unit, The Upjohn Company, Kalamazoo, Mich.

[7]We wish to thank Mrs. Susan Miedzianowski, the drug team of The Grace Hospital, and Miss Jean Devos for their dedication in dealing with this technically very demanding study.

[8]Broder LE, and Carter SK. Clinical brochure: 5-azacytidine (NSC-102816). Prepared by the NCI, Bethesda, Md, Oct 1970, 25 pp.

[9]Li LH, Buskirk HH, and Hanka LJ. Unpublished data.

[10]Neil GL. Personal communication.

[11]Ben Venue Laboratories (project No. 73-307).

ous infusion for 5 days with these objectives: to eliminate undesirable side effects (mainly nausea and vomiting) and to improve biologic activity of the drug.

## MATERIALS AND METHODS

Eight of 11 patients with various disseminated neoplasms were evaluable for toxicity. No patient had previous exposure to 5-azacytidine and all previous chemotherapy and radiotherapy courses were completed at least 4 weeks before entering this study.

Patients had adequate bone marrow function (a peripheral absolute granulocyte count of > 2000 cells/mm$^3$, a platelet count of > 100,000 cells/mm$^3$, and a normal bone marrow cellularity), adequate liver function (bilirubin < 1.0 mg/100 ml), and adequate renal function (creatinine of < 1.5 mg/100 ml). Their life expectancy was at least 8 weeks.

The drug was reconstituted with Ringer's lactate solution at a concentration of 2.5 mg/ml. The total daily dose was infused as a 24-hour continuous infusion using an EMS[12] or an IVAC[13] pump and 12-inch intracatheter. The total daily dose was divided into six 4-hour increments, each prepared freshly just prior to the infusion. The course consisted of 5 days of infusion.

There were 11 courses given at the 150-mg/m$^2$/day level and three courses given at the 170-200-mg/m$^2$/day level. Courses were repeated beginning on Day 29 or after hematologic recovery. The starting dose was selected after reviewing clinical experience from previous trials where bolus administration was used. The blood counts were done biweekly and continued daily when myelosuppression was detected.

## RESULTS

Table 1 shows the diagnoses, previous therapy, and number of courses administered. Table 2 shows a summary of hematologic toxicities. At the 150-mg/m$^2$ dose level, moderate leukopenia occurred after 18 days, which was relatively late. There was thrombocytopenia in only three courses. This was of moderate degree with an earlier start (12 days) and rapid recovery (3 days). Leukopenia was found in nine courses and in five of these it was selective neutropenia with a relative increase in lymphocyte counts. There were no changes in hemoglobin levels.

Significant toxicity was encountered in patient No. 7 who had been exposed to multiple chemotherapy and total nodal radiation. The patient developed thrombocytopenia and leukopenia on the 5th day of chemotherapy and died of septicemia on the 10th day with platelet and white blood cell counts of 7000 and 700 cells/mm$^3$ respectively.

There were two patients who showed central nervous system (CNS) abnormalities while receiving 5-azacytidine. The clinical picture was that of somnolence, extreme apathy, and at times disorientation and agitation. There were serial electroencephalogram (EEG) examinations done and diffuse abnormalities were detected. Clinical and EEG findings subsided when the drug was discontinued. No other changes could be found to account for the CNS disturbances. Both myelosuppression and CNS symptoms were clinically easily manageable and did not require premature termination of the course. One patient was treated by hyperalimentation simultaneously and the results of electrolyte determination are difficult to correlate with the chemotherapy. There were no electrolytes abnormalities in the second patient.

There was *no vomiting* and only occasional mild nausea which did not require any medical management. No diarrhea, gastroenteritis, fever, hypotension, or muscle pain was observed.

In patients No. 5 and 7 we noticed antitumor activity that could be easily defined as partial remission. Patient No. 5 had hepatic metastasis from colorectal carcinoma. Hepatic size below the costal margin decreased by 33%. This decrease in liver size could not be shown by liver scan which remained essentially unchanged for 3 months during chemotherapy. Patient No. 7 had > 50% reduction in measurable skin metastasis from a disseminated Burkitt's lymphoma.

In two patients, multiple samples of plasma and urine were analyzed by Dr. Neil using a microbiologic assay. The assay sensitivity, 0.47 μg/ml, probably prevented detection of 5-azacytidine in the plasma since no activity was found in any sample. There was a clearly detectable concentration of free 5-azacytidine in both patients' urine collection. The collections consisted of four 24-hour specimens and the total amount of free drug recovered was 0.89% in the first patient and 0.74% in the second.

## DISCUSSION

After the initial enthusiasm 5-azacytidine has been found to be a very toxic drug when administered in a bolus dose. In many reports, the side effects (nausea, vomiting, diarrhea, myelosuppression) were limiting factors in its use. Nausea and vomiting especially were very poorly tolerated by patients. In addition, there were quite significant differences in the clinical studies with respect to

[12]Electro Mechanical Specialties, Inc, Utica, Mich.

[13]IVAC Corp, San Diego, Calif.

TABLE 1.—Diagnoses, previous therapy, and number of courses given

| Patient No. | Age (yrs) | Diagnosis | Previous therapy | No. of courses of current therapy |
|---|---|---|---|---|
| 1 | 45 | Hypernephroma (clear cell adenocarcinoma) | Cobalt 60 | 2 |
| 2 | 59 | Squamous cell bronchogenic carcinoma | Methyl-CCNU, vincristine + bleomycin | 2 |
| 3 | 67 | Lymphocytic lymphoma | Cyclophosphamide, vincristine, adriamycin, prednisone | 2 |
| 4 | 28 | Lung adenocarcinoma (poorly differentiated) | Cobalt 60 (pleura + brain) 5-Fluorouracil, methyl-CCNU, adriamycin, methotrexate | 2 |
| 5 | 64 | Sigmoid colon adenocarcinoma | 5-Fluorouracil, mitomycin C, vincristine, bleomycin | 3 |
| 6 | 51 | Small cell lung carcinoma | None | 1 |
| 7 | 45 | Lymphoma (poorly differentiated, possibly American Burkitt's) | Total nodal irradiation (1/74) Cyclophosphamide, COPP* | 1 |
| 8 | 48 | Colon adenocarcinoma | 5-Fluorouracil, mitomycin C | 1 |

*COPP = cyclophosphamide, vincristine, prednisone, and procarbazine.

TABLE 2.—Summary of hematologic toxicity (leukopenia)

| | Dosage group | |
|---|---|---|
| | 150 mg/m² | 170-200 mg/m² |
| Nadir (cells × $10^3$/mm³) | | |
| Mean | 2.6 | 1.6 |
| Median | 2.8 | 1.2 |
| Range | 0.7-6.1 | 1.2-1.9 |
| Days to nadir | | |
| Mean | 17 | 21 |
| Median | 18 | 16 |
| Range | 10-25 | 16-26 |
| Days to recovery (>3.0 cells × $10^3$/mm³) | | |
| Mean | 6 | 3 |
| Median | 6 | 3 |
| Range | 2-13 | 3-4 |

tumor responses. Some studies found 5-azacytidine to be very disappointing in solid tumors (16) while others were encouraging (17). However, there was a general agreement that the drug is promising in the treatment of acute leukemias (9), again with side effects (nausea and vomiting) as limiting factors.

The findings about the stability of 5-azacytidine[9,11] had prompted us to further investigate the drug in a setting where undesirable side effects would be minimized. Our study showed that using a 24-hour infusion regimen virtually eliminates these side effects while still preserving biologic activity. The solution of the drug that was prepared in exactly the same way as in a clinical setting and was tested by microbiologic assay at different temperatures[14] showed 100% activity after 6 hours. The dose of 150 mg/m² given for 5 days seems to be optimal. We hope that our favorable results will revitalize interest in this promising drug. The preliminary results of our phase II study using the same schedule are favorable.

Recent findings that 5-azacytidine crosses the blood-brain barrier (18), the possibility of using the drug for a local organ perfusion (with the antidote given simultaneously), as well as some most recent indications about the possibility for oral administration (13) certainly open a wide field for further laboratory and clinical studies.

[14]Li LH. Biological evaluation of the stability of 5-azacytidine. Unpublished data.

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# Treatment of Small Cell Carcinoma of the Lung Using Methyl-CCNU (NSC-95441) Combined With Cyclophosphamide (NSC-26271) and Vincristine (NSC-67574) in a 3-Week Schedule[1,2]

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## SUMMARY

**Twenty-six patients with small cell carcinoma of the lung were treated with a combination of methyl-CCNU (75 mg/m² orally), cyclophosphamide (750 mg/m² iv), and vincristine (1.4 mg/m² iv) once every 3 weeks and followed for 2-56 weeks (mean, 24 weeks). An average of seven treatments were given per patient. Myelotoxicity was mild to moderate with no white blood cell count (wbc) <1000 cells/mm³ and no platelet count <25,000 cells/mm³. Six patients (23%) had a wbc of 1000-2000 cells/mm³ and two (8%) had a platelet count of 25,000-75,000 cells/mm³. Sixty-eight percent of the projected dose of methyl-CCNU was given. Fourteen of 22 patients with measurable disease (54%) responded. Of 14 patients who had received no prior treatment 64% responded with a median survival duration of 40 weeks. Complete responses occurred only in patients without prior radiation therapy or chemotherapy. We conclude that methyl-CCNU may be given with an acceptable level of toxicity in an every-3-week schedule and that the combination of cyclophosphamide, methyl-CCNU, and vincristine warrants further evaluation in the treatment of small cell carcinoma of the lung.**

[Cancer Chemother Rep 59:1127-1130, 1975]

Despite its well-known growth characteristics and its sensitivity to a large number of chemotherapeutic agents, small cell carcinoma of the lung has not responded dramatically to combination chemotherapy. Preliminary results from the Eastern Cooperative Oncology Group (ECOG)[6] demonstrate greater effectiveness with a combination of cyclophosphamide and CCNU, with 45% of the patients responding partially or completely, compared to a 28% response rate using cyclophosphamide alone in a randomized trial. Maurer et al (1) reported an enhanced effect from the combination of cyclophosphamide, vincristine, and methotrexate compared to cyclophosphamide alone, although radiation therapy was also employed. The high initial hopes for methyl-CCNU (2,3) have not been supported in phase II trials. None of 20 patients with small cell carcinoma responded, although other investigators have reported some activity in small series (4,5).

We have used cyclophosphamide, methyl-CCNU, and vincristine together to evaluate the toxicity and to assess the antitumor efficacy. Of particular interest was the use of methyl-CCNU in an every-3-week regimen, since studies with CCNU have suggested that this schedule allows a larger amount of drug to be given with less severe myelosuppression (6,7).

## METHODS

All patients with histologically or cytologically confirmed small cell lung carcinoma were eligible for this study provided their serum creatinine level was <2.0 mg/100 ml and their bilirubin level was <2.0 mg/100 ml. Patients who had received prior radiotherapy or chemotherapy were eligible if disease progression had been documented and hematologic

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[6]Combination chemotherapy for lung cancer. *In* Minutes of ECOG meeting, J. H. Edmonson, chairman. Jasper, Alberta, Canada, June 1974.

recovery had occurred. All patients were required to have a treatment-free interval of at least 6 weeks before the current therapy was started, with the exception of two patients with documented CNS metastasis who were receiving prednisone.

Treatment consisted of cyclophosphamide (750 mg/m$^2$ iv), methyl-CCNU (75 mg/m$^2$ orally), and vincristine (1.4 mg/m$^2$ iv, not to exceed 2.0 mg). All drugs were given once every 21 days. The doses of methyl-CCNU and cyclophosphamide were reduced by 50% if the white blood cell count (wbc) was <5000 cells/mm$^3$ or the platelet count was <125,000 cells/mm$^3$ at the time of therapy. If the wbc was <3000 cells/mm$^3$ or the platelet count was <75,000 cells/mm$^3$ treatment was deferred until the counts rose to a level permitting at least a 50% dosage of methyl-CCNU and cyclophosphamide.

Patients were evaluated every 3 weeks with physical examination and disease measurement including chest roentgenograms, complete blood cell counts, and platelet counts. A blood chemistry profile was obtained every 6 weeks. Measurable disease included palpable masses, abnormal chest roentgenographic densities, and hepatomegaly extending greater than 4 cm below the costal margin provided serum enzyme abnormalities and liver scan were consistent with metastatic tumor or a liver biopsy was positive.

Response to treatment was defined as ≥ 50% reduction in tumor size measured as the sum of the products of the largest diameter and widest perpendicular of each mass and/or a 50% reduction in liver size as measured from the right costal margin and the xiphoid with no evidence of progression of other lesions or development of new disease. Lesser tumor shrinkage was not considered a response but patients continued to receive treatment until obvious progression occurred. Patients were considered to have progressive disease if a new lesion developed or a > 25% increase in tumor size occurred.

## RESULTS

Twenty-seven patients were entered between October 1973 and December 1974 with an average treatment duration of 24 weeks. There were 22 men and five women with an age range of 42-76 years (mean, 62 years). One patient received one course of therapy and then refused further treatment or followup for personal reasons. Although his local physician felt he had a good tumor response with no evidence of toxicity, no measurements were recorded and he was not included in the evaluation.

Eight patients had received prior radiation therapy with or without chemotherapy and were given the current treatment upon evidence of disease progression (five had received prior radiation therapy to the chest, and three had received prior radiotherapy for symptomatic chest or brain disease and then treatment with either mechlorethamine or cyclophosphamide plus prednisone or cyclophosphamide, CCNU, and prednisone respectively). Eighteen patients had received no prior treatment. Seven of these were treated with radiation therapy to the mediastinum, neck, and involved lung at the start of chemotherapy as part of an ongoing combined treatment program. Other measurable disease was present in three patients, but four had no other measurable disease and so were not included in the evaluation of tumor response. Three of these patients died of recurrent cancer at 16, 17, and 56 weeks while the other patient is currently alive at 56 weeks but with progressive disease. These four patients are included in the toxicity and survival evaluations only.

### Tumor Response

Of 22 patients with measurable disease, 12 (54%) responded with four (18%) complete and eight (36%) partial responses. Remissions lasted 3-39+weeks (median, 21 weeks) with only one patient currently continuing in remission. Three of the eight patients who had received prior therapy responded (including two of the three patients treated with prior chemotherapy); responses tended to be short however (3, 9, and 18 weeks respectively) and were not complete. The single patient previously treated with a nitrosourea failed to respond (table 1).

Eighteen patients had received no prior therapy and 14 of these were evaluable for chemotherapy response. Nine patients (64%) achieved remission: three (21%) complete and six (43%) partial. Median survival measured from the start of chemotherapy was 40 weeks (range, 2-60 weeks) for the entire group of 18 patients. Four patients are still alive but all except one have progressive disease.

TABLE 1.—Response to therapy

| Prior therapy | No. of patients treated | No. of responses | |
|---|---|---|---|
| | | Partial | Complete |
| None | 14 | 5 | 4 |
| Radiotherapy | 5 | 1 | |
| Radiotherapy + chemotherapy | 3 | 2 | |
| Total | 22 | 8 (36%) | 4 (18%) |

Partial responses were evident by 3 weeks in all responders except one who responded by 6 weeks. Maximal response was often not evident for several additional weeks.

## Drug Toxicity

A total of 188 treatments in 26 patients were evaluated for drug toxicity. A mean of 7.2 courses were administered to all patients with 7.8 courses completed by responders and 4.2 by nonresponders. Of the total dose planned to be given, 76% of methyl-CCNU, 79% of cyclophosphamide, and 96% of vincristine was administered with an average interval between treatments of 23.3 days. Thus, the actual dosage was 68% of that planned for methyl-CCNU, 71% for cyclophosphamide, and 87% for vincristine.

Hematologic toxicity was mild to moderate in all patients (table 2). The mean nadir was 3000 cells/mm$^3$ with a low of 1200 cells/mm$^3$ for leukopenia and 155,000 cells/mm$^3$ with a low of 50,000 cells/mm$^3$ for thrombocytopenia.

Twenty-eight courses (15%) were delayed for 1-3 weeks until recovery because of a wbc <3000 cells/mm$^3$ or a platelet count <75,000 cells/mm$^3$. Treatment was withheld in 11% of the patients for the second course (at 3 weeks) and in 23% of the patients for the sixth course.

Gastrointestinal toxicity consisted of nausea and vomiting and was mild to moderate. Neurotoxicity was mild, as anticipated from the low dose of vincristine used.

TABLE 2.—Hematologic toxicity

| | Responders | Nonresponders | Total* |
|---|---|---|---|
| No. of patients | 12 | 10 | 26 |
| No. of courses (mean) | 7.8 | 4.2 | 7.2 |
| Wbc count (cells/mm$^3$) | | | |
| ≤3000 > 2000 | 7 | 4 | 12 (46%) |
| ≤2000 >1000 | 3 | 2 | 6 (23%) |
| Platelet count (cells/mm$^3$) | | | |
| ≤125,000 > 75,000 | 5 | 2 | 8 (31%) |
| ≤ 75,000 > 25,000 | | 2 | 2 (8%) |

*Includes 4 patients followed on therapy without measurable tumor.

## DISCUSSION

A limiting factor in the usefulness of the nitrosoureas is their delayed hematologic toxicity with occasionally prolonged thrombocytopenia and leukoia. We have observed (and others have reported) some patients who sustain unpredictably severe myelosuppression with initial treatment (8). This treatment program was begun to evaluate the toxicity of methyl-CCNU given in half doses every 3 weeks in combination with cyclophosphamide and vincristine with the hope that the unpredictably severe, delayed myelotoxicity from the usual 6-week course might be aborted. Fear that such a schedule might lead to a more prolonged and severe myelosuppression due to the administration of a second dosage of the drug at 3 weeks, a time before which hematologic toxicity would be evidenced in the peripheral blood counts, has not materialized.

With 188 courses administered to 26 patients and an average followup of 24 weeks on study, no complications requiring hospitalization and no drug-related deaths have occurred. The major toxicity was hematologic. One patient (7%) sustained severe toxicity with a wbc of 1200 cells/mm$^3$. Thrombocytopenia <50,000 cells/mm$^3$ was not encountered despite the use of 75% of the usual single-agent dose for both cyclophosphamide and methyl-CCNU. This compares favorably to the 23% severe and life-threatening toxicity from a combination of cyclophosphamide (70% of usual dose as a single agent) and CCNU (54% of the usual dose as a single agent given every 6 weeks) utilized by the ECOG.[7] Since the median duration of therapy for that study was 12 weeks a difference in toxicity cannot be explained on the basis of a difference in treatment duration. Myelosuppression evident 3 weeks after the initial treatment led to dose reductions in 30% of the patients and delays in the second treatment in an additional 11%. This scheduling may have been responsible for the absence of more severe toxicity.

Despite this dosage modification, 68% of the projected methyl-CCNU dose was administered. A comparative study is needed to determine whether a greater total amount of drug can be given on a 3- or a 6-week schedule and whether any difference in antineoplastic effect exists.

The possibility that the addition of vincristine might have decreased the methyl-CCNU toxicity cannot be excluded. A retrospective comparison of BCNU versus BCNU plus vincristine in the treatment of primary brain tumors found the single agent more effective, suggesting that vincristine might have an inhibiting action on the nitrosourea that could conceivably also affect bone marrow toxicity (9). No substantiation of this report has yet appeared.

The 54% objective response rate in small cell carcinoma of the lung is felt to warrant further study.

[7] See footnote 6.

Three of the six patients who had received prior radiotherapy (one also received cyclophosphamide and one mechlorethamine) responded to the combination after progression had occurred. Of the 14 patients with measurable disease who had received no prior treatment, nine (64%) achieved complete or partial remission. These results have led to the development of a randomized study comparing treatment with this drug combination alone to radiation therapy plus the combination in previously untreated patients.

## LIST OF COMPOUNDS

**BCNU:** NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1- nitroso-

**CCNU:** NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

**Cyclophosphamide:** NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

**Methotrexate:** NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

**Methyl-CCNU:** NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

**Prednisone:** NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,-11,20-trione, 17,21-dihydroxy-

**Vincristine:** NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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# Combination Chemotherapy for Acute Nonlymphoblastic Leukemia in Adults[1,2]

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## SUMMARY

**Between January 1973 and February 1975, 77 adults with acute nonlymphoblastic leukemia were treated with a combination of daunorubicin, cytosine arabinoside, 6-thioguanine, prednisone, and vincristine in university-affiliated and private institutions. After 31 patients were treated (regimen 1) the doses of all drugs were significantly increased (regimen 2). Regimens 1 and 2 yielded CR rates of 59% (17 of 29 patients) and 70% (32 of 46 patients) respectively. With regimen 2 the mean number of courses and the median number of days to CR decreased from 3 to 1.4 and from 46 to 29 respectively. Failure to achieve CR was due to persistent leukemia during regimen 1 and fatal infections during regimen 2. With regimen 2 ten of 20 patients (50%) > 50 years had CR compared to 22 of 26 patients (85%) < 50 years. CR rates were similar in community and university institutions.**

[Cancer Chemother Rep 59:1131-1137, 1975]

Advances in the treatment of leukemia have evolved in specialized centers studying disease processes, supportive care techniques, and treatment modalities. However, all patients need not and cannot be referred to specialized centers. Many prefer to be treated near their homes for personal or social reasons. It is therefore important to evaluate how well advances in leukemia therapy can be used in the community so that these patients are assured of receiving the best available therapy.

Remission induction rates in adults with acute leukemia reflect the effectiveness of chemotherapy in decreasing the tumor load and of supportive care in preventing fatal hemorrhagic and infectious complications during the induction phase. The higher remission rates observed in the past 5 years reflect advances in chemotherapy and in supportive techniques, especially platelet transfusions and more effective antibiotics.

The introduction of daunorubicin and cytosine arabinoside has had a marked impact on the therapy of acute nonlymphoblastic leukemia in adults. Daunorubicin, the most active single agent, rapidly induces CR in 35%-55% of the patients treated (1,2). However, it causes prolonged aplasia. Cytosine arabinoside induces CR in 25%-35% of the patients treated but usually more slowly and only after several courses of therapy have been given (3,4). Combinations which have included daunorubicin or cytosine arabinoside or both have induced CR rates of 55%-65% (5-11). These results have been achieved

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predominantly at leukemia centers or in cooperative groups. The efficacy of intensive therapy of acute leukemia in adults in the community has not yet been clearly established, although in one study patients > 50 years responded similarly to modest chemotherapy whether given at university centers or at community hospitals (12).

This report deals with the efficacy of two closely related intensive five-drug combination chemotherapy regimens for remission induction in 77 adults with acute nonlymphoblastic leukemia at both university and private institutions in the state of Washington. There was close communication between the physicians in practice and the university physicians, usually by telephone or at monthly meetings. Platelet support was available to all patients. Granulocyte transfusions and ultraisolation procedures were not utilized.

The primary purpose of the study was to assure the best available therapy to patients in the community via a collaborative effort between physicians within the university and those outside and to evaluate the efficacy of such therapy.

## PATIENTS AND METHODS

### Patients

From September 1972 to February 1975, 77 adults with acute nonlymphoblastic leukemia were treated. None had received prior chemotherapy other than hydroxyurea and/or prednisone. No patient was denied entry in the study because of age or poor condition, but patients dying from pre-existing sepsis during the first 24 hours of therapy were excluded from evaluation. Table 1 shows the clinical characteristics of the patients on both regimens. There was no significant difference between the patients on regimens 1 and 2. Four patients on regimen 1 and six on regimen 2 had peripheral blood blast cell counts >100,000 cells/mm$^3$. The number of patients treated at the university-affiliated hospitals decreased from 14 of 31 patients in the first regimen to seven of 46 in the second.

*Induction therapy.*—Regimen 1 (fig 1) consisted of cytosine arabinoside (2 mg/kg iv every 12 hours) plus 6-thioguanine (2 mg/kg orally every 12 hours) and prednisone (1 mg/kg orally daily), all given for 5 consecutive days, plus daunorubicin (1.5 mg/kg) and vincristine (1 mg/m$^2$) given iv on Day 1 only. Regimen 2 (fig 2) consisted of the same doses of cytosine arabinoside, 6-thioguanine, and prednisone but for 7 consecutive days, daunorubicin given at a dose of 1.5 mg/kg daily for 3 days, and vincristine given at a dose of 1 mg/m$^2$ on Days 1 and 7. Induction courses of regimens 1 and 2 were repeated 7 days after the completion of the previous course. However, if the marrow at that time was aplastic or hypoplastic with < 10% blast cells, all drugs were with-

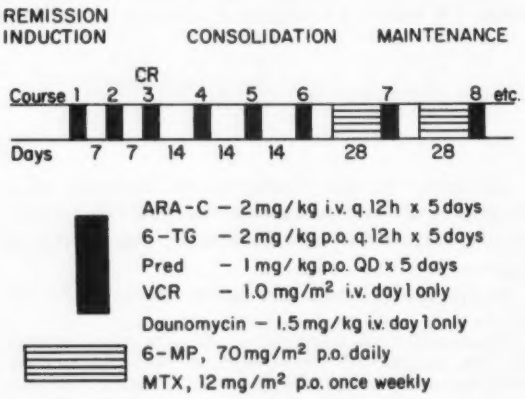
REMISSION INDUCTION
CONSOLIDATION
MAINTENANCE
CR
Course 1 2 3 4 5 6 7 8 etc.
Days 7 7 14 14 14 28 28
ARA-C – 2 mg/kg i.v. q. 12 h x 5 days
6-TG – 2 mg/kg p.o. q. 12 h x 5 days
Pred – 1 mg/kg p.o. QD x 5 days
VCR – 1.0 mg/m² i.v. day 1 only
Daunomycin – 1.5 mg/kg iv. day 1 only
6-MP, 70 mg/m² p.o. daily
MTX, 12 mg/m² p.o. once weekly

FIGURE 1.—Dose schedule for regimen 1.

TABLE 1.—Patient characteristics

| | Medians and ranges for— | |
|---|---|---|
| | Regimen 1 (31 patients) | Regimen 2 (46 patients) |
| Age | 35 (19-69; 12 > 50 yrs) | 41 (16-84; 20 > 50 yrs) |
| Hematocrit level (%) | 25 (16-35) | 27 (18-39) |
| White blood cell count (cells/mm$^3$) | 7800 (1100-188,000; 4 > 100,000) | 8300 (850-120,000; 6 > 100,000) |
| Granulocyte count (cells/mm$^3$) | 600 (50-4000) | 500 (100-7500) |
| Platelet count (cells/mm$^3$) | 40,000 (10,000-110,000) | 31,000 (18,000-120,000) |

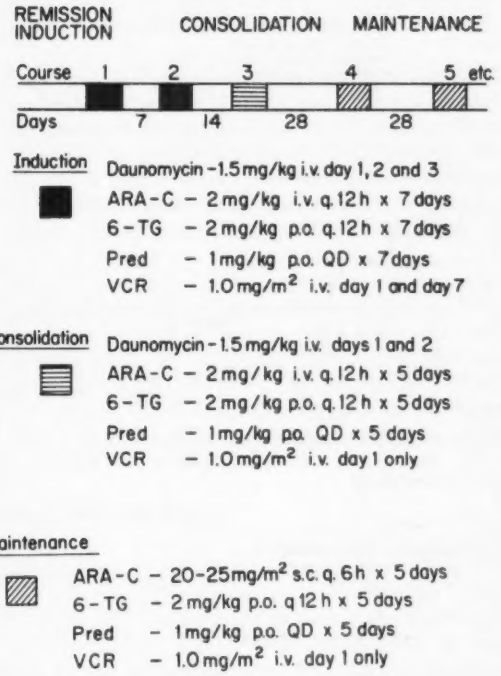
REMISSION INDUCTION
CONSOLIDATION
MAINTENANCE
Course 1 2 3 4 5 etc.
Days 7 14 28 28
Induction
Daunomycin - 1.5 mg/kg i.v. day 1, 2 and 3
ARA-C - 2 mg/kg i.v. q. 12 h x 7 days
6-TG - 2 mg/kg p.o. q. 12 h x 7 days
Pred - 1 mg/kg p.o. QD x 7 days
VCR - 1.0 mg/m² i.v. day 1 and day 7
Consolidation
Daunomycin - 1.5 mg/kg i.v. days 1 and 2
ARA-C - 2 mg/kg i.v. q. 12 h x 5 days
6-TG - 2 mg/kg p.o. q. 12 h x 5 days
Pred - 1 mg/kg p.o. QD x 5 days
VCR - 1.0 mg/m² i.v. day 1 only
Maintenance
ARA-C - 20-25 mg/m² s.c. q. 6 h x 5 days
6-TG - 2 mg/kg p.o. q 12 h x 5 days
Pred - 1 mg/kg p.o. QD x 5 days
VCR - 1.0 mg/m² i.v. day 1 only

FIGURE 2.—Dose schedule for regimen 2.

held until the benign or leukemic nature of the marrow was determined by repeated marrow examinations. If by 7 days after the first course there was no significant change in peripheral marrow blast cell counts the doses of cytosine arabinoside and daunorubicin were increased by 25%. Therapy with regimen 1 was considered a failure if the peripheral blood blast cell counts did not decrease after two courses of therapy or if a CR was not achieved after the fifth course. Regimen 2 was considered a failure if blast cell counts did not decrease after one course of therapy. Only one patient achieved CR after a third course. She remained in CR for 105 days and is evaluated with the complete responders.

*Consolidation therapy.*—Once CR was induced in regimen 1 three additional courses of the induction regimen were given at 14-day intervals. In regimen 2 consolidation therapy was reduced to one course which included 2 days of daunorubicin.

*Maintenance therapy.*—In regimen 1 maintenance therapy consisted of the same five-drug combination given every 28 days. In addition, 6-mercaptopurine (6-MP) (70 mg/m² orally daily) and methotrexate (12 mg/m² orally once weekly) were given between these courses. In regimen 2 maintenance therapy consisted of cytosine arabinoside (2 mg/kg sc), 6-thioguanine (2 mg/kg orally), and prednisone (1 mg/kg orally), all given daily for 5 days, and vincristine (1.0 mg/m²) on Day 1 of each 28-day cycle. Daunorubicin given at a dose of 1.5 mg/kg/day iv for 2 days was added to the fourth, eighth, and 12th courses in which cytosine arabinoside, 6-thioguanine, and prednisone were decreased to 3 days.

### Supportive Care

All patients received allopurinol. If the initial serum uric acid level was high, diamox was given and a high alkaline urine output was maintained. Platelet concentrates were administered to maintain a platelet count > 20,000 cells/mm³. Broad spectrum antibiotics, Keflin and gentamicin or carbenicillin and gentamicin, were given for fever > 101°F and continued until recovery of the granulocyte count to >1000 cells/mm³.

### Evaluation and Criteria for Remission

Pretherapy studies included complete blood cell count (cbc), marrow aspiration, chest roentgenograms, liver function tests, calcium, uric acid, electrolytes, and blood urea nitrogen or creatinine.

Studies during therapy included a cbc at least three times a week and renal and liver function tests at least once weekly and more often if clinically indicated. Marrow aspiration was repeated 7 days after the end of each course of therapy unless blast cells persisted in the peripheral blood. A marrow aspiration was also obtained before each course of consolidation and maintenance therapy.

A CR was defined as a marrow containing < 5% blast cells in the presence of a peripheral granulocyte count > 1500 cells/mm³, a platelet count > 100,000 cells/mm³, a hematocrit level > 30%, and no other evidence of leukemia. No distinction was made between partial remission and failure—both were classified as failures.

## RESULTS

### Remission Rates

Thirty-one patients were treated on regimen 1. Two patients died of bacterial sepsis within 24 hours and were excluded from evaluation. Seventeen of the 29 evaluable patients achieved CR (table 2). The median time to CR was 46 days and the mean number of courses to CR was three. The main reason for failure was the resistance of the leukemia to chemotherapy. Of the 12 patients failing to attain CR, ten had leukemia resistant to chemotherapy and died after

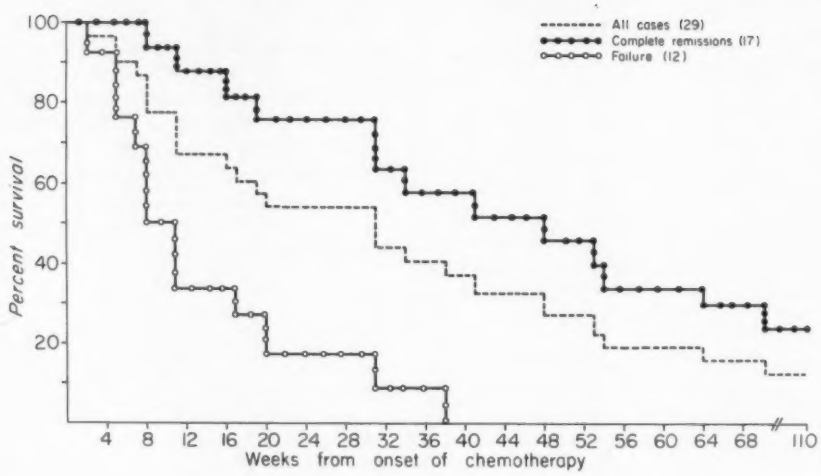


FIGURE 3.—Survival in wks from time of diagnosis with regimen 1.

TABLE 2.—Results of therapy

| | Regimen 1 | Regimen 2 |
|---|---|---|
| Total No. of patients treated | 31 | 46 |
| No. of patients evaluable | 29 | 46 |
| No. of patients with CR (%) | 17 (59) | 32 (70) |
| Mean No. of courses to CR (range) | 3 (1-5) | 1.4 |
| Median No. of days to CR (range) | 46 (17-73) | 27 (7-80) |

receiving two or more courses of therapy, while two patients, both > 65 years, died of infection before receiving an adequate trial. During induction therapy, all patients required platelet transfusions and almost all received broad spectrum antibiotics for fever. Thus, in regimen 1 the CR rate was 59%, the time to remission was long, and the main reason for failure was resistance of leukemia to the drugs.

Forty-six patients received regimen 2 and all were evaluable. Thirty-two of 46 patients (70%) achieved CR (table 2). The median time to CR was 27 days and the mean number of courses to CR was 1.4. In contrast to regimen 1, the main reason for failure to achieve CR was fatal infection. Of 14 patients failing to attain CR, ten (eight patients > 50 years old) died of infection before receiving an adequate trial of induction therapy and only four patients had leukemia resistant to the regimen. All patients required platelet transfusions and almost all received broad spectrum antibiotics for fever or documented sepsis.

On both regimens only two of 43 patients < 50 years old died without an adequate trial of therapy (table 3). Thus, in the younger patients the main impediment to CR induction was resistance to antileukemic therapy. Regimen 2 was more effective for patients <50 years old than regimen 1 with 22 of 26 patients (85%) and nine of 17 patients (53%) achieving CR respectively. In contrast, the main impediment to CR induction in patients > 50 years was the inability to tolerate aplasia and associated infections. In this instance regimen 2 was no more effective than regimen 1. Among patients > 50 years four of 12 patients and ten of 20 patients on regimens 1 and 2 respectively failed to achieve CR. Two of the four patients and eight of the ten patients died of infection before receiving an adequate trial of induction therapy.

With both regimens remission induction rates were similar at university-affiliated and private institutions. With regimen 1, eight of 13 patients treated at the university and nine of 16 treated in the community achieved CR. Corresponding results with regimen 2 were six of seven patients and 26 of 39 patients respectively. Nineteen of 39 patients treated in private institutions were > 50 years as was one of seven in university-affiliated institutions.

## Remission Duration and Survival Data

*Regimen 1.*—The median remission duration in all 17 patients achieving CR, including three who were not given maintenance therapy, was 22 weeks. Seven had remissions > 46 weeks and four of these are still in CR at 71+-113+ weeks. All who failed to achieve CR died with a median survival time of 8 weeks. The median survival time for patients who attained CR was 47 weeks (fig 3).

*Regimen 2.*—Although it is too early to determine the median duration of CR, the data available (table 4) show that it is > 7 months. All the patients who failed therapy died within 12 weeks after therapy started. Among the patients < 50 years, 22 of 26 are still alive at 14+-51+ weeks. No patient on either protocol had central nervous system leukemia as the first manifestation of relapse.

## Patient Accrual Rates

Thirty-one patients were entered on regimen 1 in 18 months and 46 were entered on regimen 2 in 8 months. The increased accrual rate is due to the fact that most hematologists and oncologists in this state are using the protocol now while only about 30% were participating during the first study.

TABLE 3.—Results of therapy in patients < 50 years of age

| | Regimen 1 | Regimen 2 |
|---|---|---|
| No. of patients treated | 17 | 26 |
| No. of patients evaluable | 17 | 26 |
| No. of patients with CR (%) | 9 (53) | 22 (85) |
| Mean No. of courses to CR (range) | 3 (1-5) | 1.7 (1-2)* |
| Median No. of days to CR (range) | 46 (17-62) | 27 (7-80) |

* 1 patient achieved CR with 3 courses.

TABLE 4.—Duration of CR with regimen 2

| | |
|---|---|
| No. of patients with CR/No. evaluable | 32/46 |
| No. of patients relapsing/No. induced | 10/32 |
| Duration of CR in patients who relapsed (days) | 89, 90, 105, 182, 186, 224, 247, 281, 298, 320 |
| No. of patients remaining in CR/No. induced | 22/32 |
| No. of patients remaining in CR > 7 mos | 11 |
| No. of patients remaining in CR < 7 mos | 11 |

## Drug Toxicity

*Regimen 1.*—During induction therapy transient marrow depression occurred in all patients. Nausea and vomiting were nonexistent or only mild in 80% of the patients and were moderate in 20%. There was no evidence of liver or renal damage due to chemotherapy. Neurologic toxicity from vincristine was minimal and alopecia was common. Consolidation therapy, routinely given on an outpatient basis, was well tolerated. No patient became infected or required platelet transfusions. Maintenance therapy in regimen 1 initially consisted of the five-drug combination with 6-MP and methotrexate administered between courses. There were three cases of sepsis among the first 20 patients, all of which were associated with granulocytopenia (< 500 granulocytes/$mm^3$). Therefore, maintenance therapy with 6-MP and methotrexate was discontinued for the remaining nine patients. The rest of the maintenance therapy, administered on an outpatient basis, was well tolerated without sepsis or hemorrhage. Subjective toxicity was mild, allowing normal activity.

*Regimen 2.*—During induction therapy the marrow depression was profound. All patients exhibited granulocytopenia of 0-200 cells/$mm^3$ for 1-3 weeks after the first course and for longer periods if two courses were required. Nausea and vomiting were comparable to that observed with regimen 1. There were four cases of transient mild hepatitis as shown by rises in bilirubin and serum glutamic oxaloacetic transaminase. Neurologic toxicity related to vincristine was limited to loss of deep tendon reflexes and mild paresthesias. Alopecia occurred in all patients. A 63-year-old patient died of congestive heart failure after daunorubicin was given at a total dose of 9.0 mg/kg during induction therapy. Consolidation therapy was well tolerated. No serious infections occurred but 30% of the patients required platelet transfusions. Most consolidation courses were given on an outpatient basis but some did require hospitalization for several days. Maintenance therapy was well tolerated with nonexistent to minimal subjective toxicity, no requirement for platelets, and no bacterial infections. Since cytosine arabinoside was given sc, only one outpatient visit per month was required.

## DISCUSSION

The introduction of daunorubicin and cytosine arabinoside has markedly improved remission induction rates in adults with acute leukemia. Either drug alone induces CR in over 30% of the patients (1-4). The availability of active single agents allows development of effective combination chemotherapy regimens. Combinations which have included cytosine

arabinoside have yielded CR rates of 50%-65% (5, 6,13) but with a long induction period. In contrast, combinations which have included optimal doses of daunorubicin have induced equivalent CR rates but more rapidly (7,9,11). Our protocol combines maximal doses of effective single agents. Vincristine and prednisone were included in our combination therapy because their toxic effects and actions differ from the more active agents. Although their use has not been proven in this regimen, they do not interfere with the administration of full doses of the more active drugs and they have some effect against adult leukemia (14). Drug dosages were increased significantly when it became evident that the time to CR was long and the incidence of persistent leukemia with regimen 1 was high. The most significant change was the threefold increase of daunorubicin. Regimen 2 (the more vigorous regimen) was still well tolerated in patients< 50 years and was associated with a higher incidence (85%) of CR induction. An accurate comparison of this five-drug regimen to the combination of optimal doses of daunorubicin plus cytosine arabinoside would require a randomized trial (8).

Therefore, the principal problem in patients < 50 years is not how to induce a higher incidence of CR but how to prolong the duration of remission. It is possible that the introduction of an additional active chemotherapeutic agent or the addition of immunotherapy to intermittent maintenance chemotherapy will significantly increase the median remission duration and yield more long-term disease-free survivors.

During induction therapy in adults with acute leukemia supportive care is essential to success. As marrow-toxic regimens have become more intense, the duration and degree of pancytopenia have greatly increased. Advances in platelet support and antibiotic therapy have decreased the incidence of fatal hemorrhage and infection during induction therapy. Less than 5% of our patients < 50 years died of infection or hemorrhagic complications before receiving an adequate trial of induction therapy. Thus, patients < 50 years were able to survive the period of aplasia associated with remission induction. In contrast, 33% of the patients > 50 years, including 13 of 20 patients > 60, died of infection before receiving an adequate trial of chemotherapy. Therefore, it is the older patients who might benefit most from newer supportive techniques such as laminar air-flow rooms, gut and skin sterilization, and granulocyte transfusions (15-19) because their major problem is to survive the period of marrow aplasia.

Approximately 50% of our patients > 50 years did attain CR with present chemotherapy regimens and had remission durations similar to those of the younger patients. There is no evidence that elderly patients have more resistant leukemia since their lower remission rate can be explained by fatal infections during induction therapy. However, while more intensive combination therapy regimens aimed at inducing remissions quickly are superior in the younger age group they are associated with a high incidence of infectious complications in elderly patients. Therefore, patients > 50 years seem to fare equally, although poorly, with any of a number of adequate regimens (5-7,9,13).

The rationale for withholding chemotherapy in elderly patients with acute leukemia is suspect. Without therapy their lifespan is short and of poor quality and they often require hospitalization. The rigors of induction therapy are difficult but self-limited, and once in CR patients may return to normal activity. With our maintenance program, subjective toxicity was minimal and only one clinic visit per month was required.

Our results, like those of Grann et al (12), suggest that adequate treatment can be administered to patients outside a special leukemia center or ward if adequate platelet support is available. Specialized leukemia wards obviously offer an optimal situation for intensive investigation of leukemia and its treatment. However, although many patients prefer to be treated at such centers, others prefer to remain close to home. This study shows that well-trained community hematologists and oncologists collaborating with university physicians can administer intensive combination chemotherapy and induce remission rates similar to those reported from various leukemia centers. The results suggest that similar collaborative arrangements might be applicable to the treatment of other neoplasms.

## LIST OF COMPOUNDS

**Allopurinol:** NSC-1390; CAS reg. No. 315-30-0; 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol

**Cytosine arabinoside:** NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

**Daunorubicin:** NSC-82151; CAS reg. No. 23541-50-6; daunomycin

**6-Mercaptopurine:** NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate; 6-MP

**Methotrexate:** NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

**Prednisone:** NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,-11,20-trione, 17,21-dihydroxy-

**6-Thioguanine:** NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-

**Vincristine:** NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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# Phase I Study of *Corynebacterium parvum* in Patients With Solid Tumors[1,2,3]

**Pierre R. Band,[4] Cataminda Jao-King, Raul C. Urtasun, and Mant Haraphongse[5,6]**

## SUMMARY

**Nineteen patients with various solid tumors were treated with *Corynebacterium parvum* for 10 consecutive days at doses ranging from 0.5 to 6 mg/m². Major toxic effects included rigors and cyanosis, hypertension, headache, nausea, and vomiting. Toxicity was maximal during the first 3 days of treatment and decreased or even disappeared when, on subsequent days, increasing doses of the vaccine were given. Objective tumor regressions were observed in four patients.**

[Cancer Chemother Rep 59:1139-1145, 1975]

The potent stimulatory effect of *Corynebacterium parvum (C. parvum)* on the reticuloendothelial system (1) and its antitumor activity in a variety of experimental tumor systems (2-5) have been described in a recent review (6).

In man, *C. parvum* given sc was shown to prolong the survival of cancer patients treated with this vaccine in addition to chemotherapy when compared to patients receiving chemotherapy alone (7). Further, objective tumor regressions in patients with advanced metastatic malignancies have been noted with *C. parvum* when given alone by the iv route (8). These observations prompted us to study the human toxicity of iv *C. parvum*.

## MATERIALS AND METHODS

### Patient Population

Nineteen patients, six men and 13 women with progressive metastatic malignancies from various sites, were studied; all but four were previously treated with, and had relapsed after, chemotherapy or irradiation therapy or both. The patients' characteristics are indicated in tables 1 and 2.

### Drug Dosage and Schedule

The heat-killed, formalin-treated preparation of *C. parvum* was supplied in 2-ml ampules containing 4 mg of *C. parvum*. The agent was administered iv at dosages ranging from 0.5 to 6 mg/m². Individual doses were mixed in 250 ml of 5% dextrose in water, infused over 1 hour, and given daily for 10 consecutive days as defined in the following schedules.

#### *Schedule A*

Constant daily doses were given to eight patients. Dosages were 0.5 mg/m² for three patients, 0.6 mg/m² for one, 1 mg/m² for two, and 2.4 mg/m² for two. It was observed that side effects were maximal during the first 3 days of therapy and subsequently decreased. In an attempt to minimize the intensity of the toxic reactions, treatment was modified as described under schedule B.

#### *Schedule B*

Eleven patients were treated. *C. parvum* was given at progressively increasing daily doses during the first 3-6 days of therapy until a maximum dose was reached and was then kept constant for the remainder of the 10-day treatment course. All but one patient received their respective highest dose of *C. parvum* for at least 5 consecutive days. Maximum doses were 2.5, 4, 4.6, and 4.8 mg/m² for each of four patients respectively, 5 mg/m² for four, and 6 mg/m² for three.

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[2]Presented in part at the meeting of the American Association for Cancer Research, San Diego, May 1975.

[3]*Corynebacterium parvum* (lot No. S0274) was supplied by the Institut Merieux, Lyon, France.

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[6]We are indebted to Dr. C. Charbonnier, Institut Merieux, Lyon, France, for the gift of *Corynebacterium parvum* used in this study, and to Dr. L. Israël for many helpful discussions.

TABLE 1.—Schedule A: patient information and predominant side effects occurring on the day toxicity to iv *C. parvum* was maximal

| Patient No. | Age (yrs)/ sex | Primary tumor site | Prior therapy* | Indicated parameters at maximal toxicity | | | |
|---|---|---|---|---|---|---|---|
| | | | | Day of treatment | *C. parvum* dose (mg/m²) | Chill phase† | Rectal temperature (°C) |
| 1 | 46/F | Breast | RT,C | — | 0.5 | 0 | 37 |
| 2 | 76/M | Stomach | C | 2 | 0.5 | 1+ | 39 |
| 3 | 28/M | Bone sarcoma | RT | 3 | 0.5 | 4+ | 40 |
| 4 | 54/F | Breast | RT,C | 2 | 0.6 | 2+ | 39.5 |
| 5 | 11/F | Ovary | RT | 2 | 1.0 | 3+ | 39.8 |
| 6 | 44/M | Pancreas | C | 2 | 1.0 | 3+ | 39 |
| 7 | 33/F | Colon | C | 1 | 2.4 | 4+ | 40.5 |
| 8 | 45/F | Colon | C | 2 | 2.4 | 4+ | 39.8 |

* RT = radiation therapy; C = chemotherapy.
† 0 = No toxicity; 1 + = shivers, no cyanosis; 2 + = mild rigors and cyanosis; 3 + = moderate rigors and cyanosis; 4 + = marked rigors and cyanosis.

Corticosteroids, antipyretic drugs, or chemotherapeutic agents were withheld during the entire treatment course.

### Patient Evaluation

Prior to and at the end of therapy all patients had a complete medical history and physical examination taken, complete blood cell (cbc) count including hemoglobin, total and differential white blood cell counts, and platelet counts, liver and renal function tests, fibrinogen, fibrin split products, direct and indirect Coombs' test, serum electrophoresis and immunoelectrophoresis, chest roentgenogram, and electrocardiogram. During therapy, patients had cbc counts taken twice weekly. Blood pressure, pulse, respiration, and rectal temperature were taken at 15-minute intervals during and for 2 hours after *C. parvum* infusions, and at hourly intervals for 6 hours after this.

## RESULTS

The most common toxic effect was a chill phase followed by fever. The chill phase, consisting of rigors heralded by a cold sensation and pallor, lasted for about an hour. Rigors were associated with cyanosis, hyperventilation, tachycardia, and, at times, hypertension. The body temperature began to rise during the chill phase and remained elevated for up to 8 hours. A second chill phase occurred 8-12 hours after the initial phase in three patients. The intensity of the chill phase and the degree of fever on the day that toxicity was maximal are indicated in tables 1 and 2.

### Schedule A (table 1)

Toxic effects were maximal during the first 3 days of treatment and decreased or subsequently disappeared. The chill phase was more intense at *C. parvum* doses $\geq$ 1 mg/m². Fever was high regardless of dose.

### Schedule B (table 2)

Similarly, toxic effects lessened after the first 3 days of treatment despite the higher *C. parvum* dosage. In all but two patients, during the constant-dose phase, toxicity was maximal on the day the maximum dose of *C. parvum* was reached.

Table 3 summarizes the toxic effects observed. Hypertension was seen in eight patients during episodes of moderate or marked rigors and cyanosis but never in their absence. A systolic pressure of 180 mm Hg or greater and a diastolic pressure of 100 mm Hg or more occurred in four and six patients respectively. With the increase in blood pressure, one patient had an attack of angina pectoris, and another patient experienced mild congestive heart failure; both recovered uneventfully. Headaches, nausea, and vomiting were common. Leukopenia (nadir, 2600 cells/mm³) and thrombocytopenia (nadir, 60,000 cells/mm³) occurred in four and five patients respectively; these hematologic manifestations were transient and reverted to normal despite continua-

TABLE 2.—Schedule B: patient information and predominant side effects occurring on the day toxicity to iv *C. parvum* was maximal

| | | | | Indicated parameters at maximal toxicity | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Increasing dose phase | | | | Constant dose phase | | | |
| Patient No. | Age (yrs)/ sex | Primary tumor site | Prior therapy* | Day of treatment | *C. parvum* dose ($mg/m^2$) | Chill phase† | Rectal temperature (°C) | Day of treatment | *C. parvum* dose ($mg/m^2$) | Chill phase† | Rectal temperature (°C) |
| 9 | 53/M | Lung | None | 2 | 1.2 | 3+ | 39.2 | 4 | 2.5 | 1+ | 38.9 |
| 10 | 49/F | Lung | RT | 2 | 2.0 | 3+ | 40.2 | 4 | 4.0 | 3+ | 38.5 |
| 11 | 51/F | Colon | C | 3 | 2.0 | 3+ | 39.4 | 6 | 4.6 | 2+ | 37 |
| 12 | 54/F | Breast | RT,C | 3 | 1.8 | 3+ | 38 | 6 | 4.8 | 1+ | 39.6 |
| 13 | 50/F | Colon | C | 1 | 2.5 | 4+ | 39 | 6 | 5.0 | 2+ | 40 |
| 14 | 32/F | Breast | C | 1 | 2.6 | 4+ | 39.9 | 6 | 5.0 | 1+ | 38.9 |
| 15 | 67/F | Pancreas | None | 1 | 0.7 | 3+ | 39 | 6 | 5.0 | 2+ | 38 |
| 16 | 66/M | Pancreas | C | 2 | 1.8 | 3+ | 39.6 | 5 | 5.0 | 2+ | 39 |
| 17 | 21/F | Soft tissue sarcoma | RT | 3 | 2.6 | 2+ | 39 | 7 | 6.0 | 3+ | 40 |
| 18 | 48/F | Pancreas | None | 1 | 1.4 | 3+ | 39 | 10 | 6.0 | 3+ | 38.4 |
| 19 | 38/M | Lung | None | 3 | 1.7 | 4+ | 40.4 | 7 | 6.0 | 2+ | 38.6 |

*RT = radiation therapy; C = chemotherapy.
†0 = No toxicity; 1+ = shivers, no cyanosis; 2+ = mild rigors and cyanosis; 3+ = moderate rigors and cyanosis; 4+ = marked rigors and cyanosis.

TABLE 3.—Summary of toxic manifestations

| Toxic effects | No. of patients with toxic effects on — Schedule A | Schedule B |
|---|---|---|
| Rectal temperature ≥ 38°C | 7 | 11 |
| Rigors and cyanosis | 5 | 11 |
| Blood pressure (≥ 140/90 mm Hg) | 3 | 5 |
| Systolic pressure (≥ 180 mm Hg) | 1 | 3 |
| Diastolic pressure (≥ 110 mm Hg) | 1 | 5 |
| Headaches | 5 | 6 |
| Nausea | 6 | 8 |
| Vomiting | 3 | 6 |
| Muscular pain | 3 | 6 |
| Back pain | 2 | 2 |
| Leg cramps | 1 | 1 |
| Second chill phase | 1 | 2 |
| Leukocytes (≤ 4000 cells/mm³) | 3 | 1 |
| Platelets (≤ 100,000 platelets/mm³) | 2 | 3 |
| Total No. of patients treated | 8 | 11 |

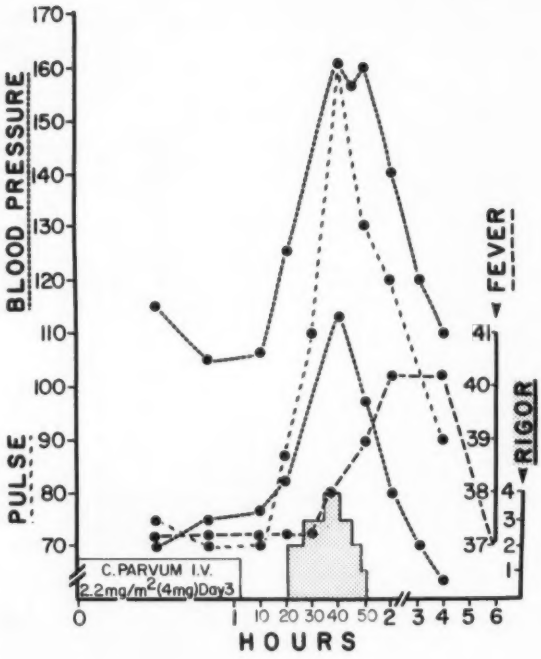


FIGURE 1.—Rise in blood pressure, heart rate, and rectal temperature during rigors induced by iv *C. parvum*.

tion of therapy. A mild and transient elevation of serum alkaline phosphatase was noted in three cases. Treatment had to be discontinued, due to toxicity, in only one patient after 8 days of therapy.

During the course of this study, four patients with measurable disease had objective tumor regressions. Patient No. 4 had a decrease of a soft tissue mass short of a partial response (50% decrease in the product of two perpendicular tumor diameters). Partial regressions of a soft tissue mass in patient No. 8 and of multiple pulmonary metastases in patient No. 3 were seen, while a dramatic decrease in liver size associated with improvement of liver function tests occurred in patient No. 11.

## CASE REPORTS

*Patient No. 19: illustration of time course of side effects.*—The hypertensive episode occurring during the chill phase and the decrease in toxicity upon repetitive *C. parvum* administration, despite dosage increase, were well demonstrated in a 38-year-old man with no evidence of cardiovascular disease. On the third day of treatment this patient was studied in the cardiac catheterization laboratory where his intrabrachial arterial pressure, heart rate, and rectal temperature were monitored. Twenty minutes after an infusion of 2.2 mg/m² of *C. parvum*, rigors began, rapidly reaching maximal intensity; at their height, the patient was markedly cyanotic with a blood pressure of 160/115 mm Hg and a heart rate of 160 beats/minute. His temperature progressively rose to 40°C where it plateaued for 2 hours before gradually declining (fig 1). On subsequent days, despite increasing *C. parvum* doses to a maximum of 6 mg/m², rigors diminished in intensity and disappeared while blood pressure remained normal (table 4).

*Patient No. 3: illustration of tumor regression.*—A 28-year-old man with multiple pulmonary metastases from an osteogenic sarcoma of the humerus received a course of iv *C. parvum* at a daily dose of 0.5 mg/m² beginning February 26, 1975. Treatment was discontinued after 8 days due to toxicity. On March 31, most of the pulmonary lesions had regressed, and an attempt at reinducing therapy was abandoned after one infusion of *C. parvum* because of recurring severe side effects. The patient remained without further therapy except for one im injection of 1 mg of *C. parvum* given on April 25. On June 10, the pulmonary metastases were no longer visible on the chest roentgenogram except for one lesion that had undergone a partial response. Figure 2 illustrates the effect of treatment on one of the pulmonary metastases.

TABLE 4.—Decrease in toxicity with time despite increasing doses of iv *C. parvum*

| Day of treatment | *C. parvum* dose ($mg/m^2$) | Chill phase* | Rectal temperature (°C) | Blood pressure (systolic/diastolic [mm Hg])† |
|---|---|---|---|---|
| 1 | 1.1 | 4+ | 39.1 | NT |
| 2 | 1.7 | 4+ | 40.4 | 180/100 |
| 3 | 2.2 | 4+ | 40.2 | 160/115 |
| 4 | 2.2 | 2+ | 39.8 | N |
| 5 | 3.3 | 2+ | 39.5 | N |
| 6 | 4.4 | 1+ | 39.2 | N |
| 7 | 6 | 2+ | 39.3 | N |
| 8 | 6 | 1+ | 38.2 | N |
| 9 | 6 | 0 | 38.2 | N |
| 10 | 6 | 0 | 38.6 | N |

*0 = No toxicity; 1+ = shivers, no cyanosis; 2+ = mild rigors and cyanosis; 3+ = moderate rigors and cyanosis; 4+ = marked rigors and cyanosis.
†NT = not taken; N = normal blood pressure (≤140/90).

## DISCUSSION

The predominant toxic effects of iv *C. parvum* were similar to those induced by an endogenous febrile reaction. Endogenous febrile reactions have been characterized by a chill phase consisting of rigors and vasoconstriction followed by a "flush phase" with fever and vasodilation (9). Depending on their intensity, the isometric muscular contractions caused by rigors, coupled with peripheral vasoconstriction, may induce a rise in blood pressure. In this study, brief but sudden hypertensive episodes were observed. These could represent a definite hazard in patients with compromised cardiovascular status.

Fever and headaches were the major toxic effects associated with the "flush phase." One patient with an ulcerated stomach cancer developed an acute upper gastrointestinal hemorrhage. Although this complication may have been coincidental, the intense vasodilation of the "flush phase" may have acted as a trigger.

A maximal tolerated dose could not be accurately defined because the degree of toxicity varied with the day of *C. parvum* administration. During the first 3 days of therapy, doses as low as 1 mg could induce severe side effects, whereas even with much larger doses tolerance to the vaccine was the rule later in the course.

Thus, treatment should be initiated at low doses in order to avoid the untoward reactions associated with the chill phase. Great caution should be exercised in patients with underlying cardiovascular disease. In particular, the iv mode of *C. parvum* administration constitutes, in our view, an absolute contraindication in patients with a history of myocardial infarction.[7]

Although this phase I trial was not designed to assess therapeutic response, our observation that iv *C. parvum* induces tumor regression in patients with advanced malignancies confirms the findings of Israël and Edelstein (8) and warrants further studies of this immunotherapeutic agent.

[7]Haraphongse M, Band PR, Basualdo C, et al. Cardiovascular changes during chills and fever induced by *Corynebacterium parvum*. In preparation.

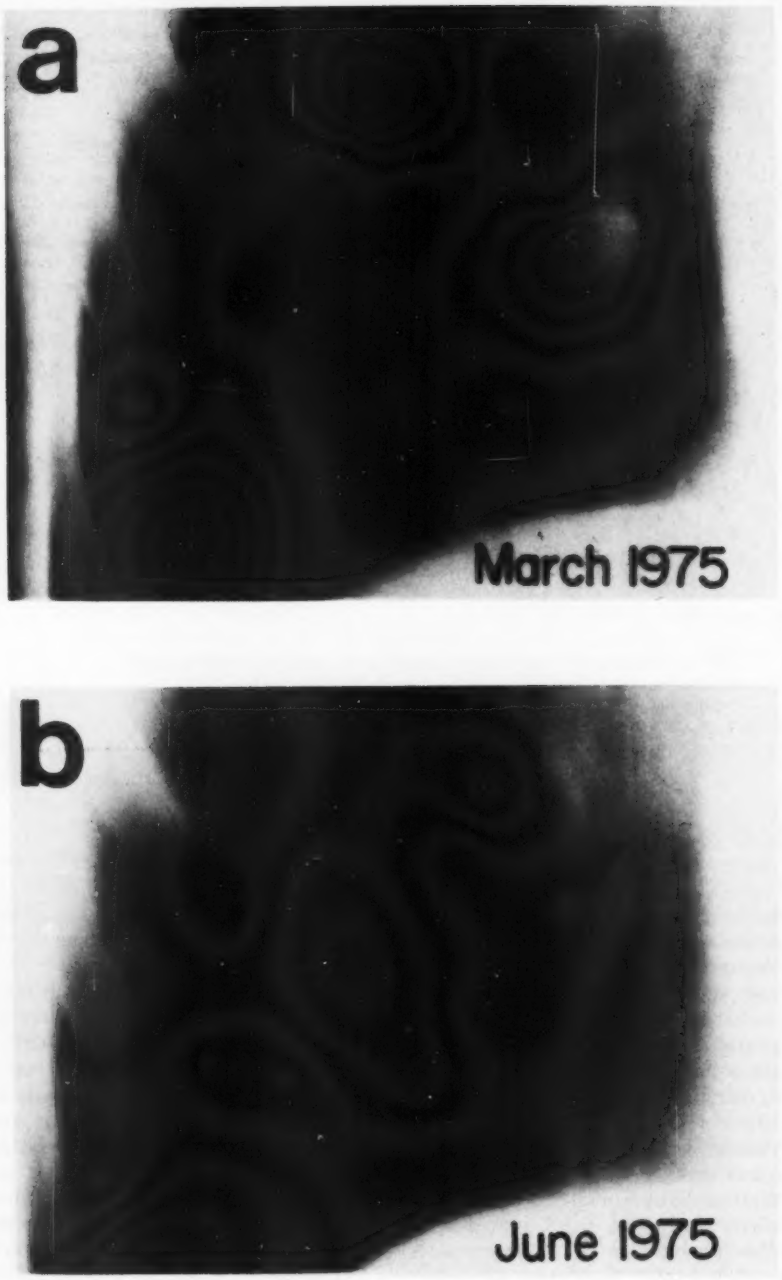


FIGURE 2.—Regression of a pulmonary metastasis in a patient with an osteogenic sarcoma. a. Roentgenogram taken on the 4th day of the first course of *C. parvum*; well-defined metastasis in right lower lobe. b. Roentgenogram taken after *C. parvum* therapy; regression of the same lesion (indicated by arrow).

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# Bleomycin (NSC-125066) and CCNU (NSC-79037) in the Combination Chemotherapy of MOPP-Resistant Hodgkin's Disease[1,2]

John E. Kurnick,[3] Madeline White, Donald E. Ware, and William A. Robinson[4]

## SUMMARY

**Twenty-two patients with MOPP-resistant stage IVB Hodgkin's disease were treated with a combination of bleomycin and CCNU. The response rate in 18 patients surviving at least 1 month was 72% with 11 partial and two complete responses. The mean durations of response and survival in partial responders were 12.2 and 17.5 months respectively. The two complete responses resulted in survivals of 20 and 36+ months. Bleomycin toxicity contributed to two deaths, one pulmonary and one hypotensive. Severe CCNU toxicity occurred after three of 82 administrations but there were no CCNU-related deaths. The majority of patients in the study tolerated the regimen without serious toxicity. Although highly effective in the temporary control of advanced resistant Hodgkin's disease, the program will hopefully be improved by the addition of longer-acting agents.**

[Cancer Chemother Rep 59:1147-1150, 1975]

The four-drug combination of mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) has proven highly effective in the induction of prolonged remissions in patients with disseminated Hodgkin's disease (1). However, approximately one third of these patients will fail to obtain an initial remission on this combination, and approximately one half of those who do enter remission will have a relapse over the succeeding 4 years (2). Thus, most centers are beginning to experience increasing numbers of patients with disseminated Hodgkin's disease no longer responsive to the conventional chemotherapeutic combinations. Further, by virtue of extensive previous myelotoxic therapy, many of these patients have markedly diminished bone marrow reserves, and this often seriously limits the ability to treat them aggressively with combinations of second-line agents.

We have attempted to overcome these difficulties by combining bleomycin, an antitumor antibiotic essentially free of myelotoxicity, with CCNU, a nitrosourea given orally manifesting greatly delayed marrow suppression. The principal toxic effect of bleomycin is to skin, mucous membranes, and lungs. It may cause severe febrile reactions within 2-6 hours of administration, but these can usually be minimized by premedication with diphenhydramine. Its most serious side effect is the production of pulmonary fibrosis with a fatality rate between 2% and 8% (3). While serial pulmonary functions are of little or no value in predicting the start of pulmonary fibrosis (3), the incidence of this complication has been noted to rise markedly at cumulative doses in excess of 400 units (4). The toxicity of CCNU consists primarily of nausea and vomiting within hours after the administration of the drug and late pancytopenia secondary to marrow depression. The white blood cell count nadir has been reported to occur on an average of 42 days after CCNU administration (5).

## MATERIALS AND METHODS

Only patients with stage IVB Hodgkin's disease who had failed or relapsed on combination chemotherapy with MOPP (1) or cyclophosphamide, vincristine, procarbazine, and prednisone (COPP) (6) were eligible for the study. A minimum absolute granulocyte count of 1500 cells/mm$^3$ and platelet

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count of 50,000 cells/mm$^3$ were also required unless the cytopenias were felt to result directly from marrow infiltration with Hodgkin's disease. The 22 patients entered in the study between April 1972 and December 1973 consisted of 14 men and eight women. Seven patients had failed to achieve remission after three to six courses of MOPP or COPP. The other 15 patients had relapsed after initial complete remissions with MOPP or COPP and failed to respond to reinstitution of the initially effective therapy.

The chemotherapy program consisted of bleomycin given iv at a dose of 0.25 units/kg twice weekly for 3 weeks followed by 7.5 units twice weekly for 2 weeks. If the patient was doing well after a total of 5 weeks the dosage was reduced to 7.5 units weekly. CCNU was given at a dose of 130 mg/m$^2$ orally every 6 weeks. Dosage was reduced to 100 mg/m$^2$ if neutropenia occurred (< 2500 cells/mm$^3$) and was omitted entirely for neutrophil counts < 1500 cells/mm$^3$ or platelet counts < 50,000 cells/mm$^3$. Complete response was defined as the disappearance of all measurable disease parameters and symptoms for at least 1 month. Partial response was defined as a reduction in the size of all measurable tumor masses by 50% or more for a similar period of time.

## RESULTS

Complete responses were achieved in two patients and partial responses in 11, for an overall response rate of 59%. In the 18 patients surviving at least 1 month the response rate was 72%. As shown in table 1, age and prior duration of disease varied widely in both responders and nonresponders. In women and in patients with nodular sclerosing histology the response rate was slightly but not significantly higher. Survival correlated directly with the duration of response. Two patients had complete clinical responses. One remission lasted 18 months before the patient died of gastric carcinoma. Only minimal evidence of Hodgkin's disease was found at autopsy. The second patient remains in complete clinical remission 36 months after therapy started. Eleven patients manifested partial responses ranging from 6 to 21 months with a mean duration of 12.2 months. These patients had a mean survival time of 17.5 months. All 11 partial responders have relapsed; eight have died, all but one within 6 months of the termination of their responses. Three patients are alive on alternate therapy programs 6, 9, and 13 months after responses of 15, 15, and 16 months respectively. All nonresponders died within 5 months of the start of treatment and four of them died in < 1 month which was an indication of the severity of the illness in many of the patients in this study.

Toxicity of the program varied greatly from patient to patient. Seven patients had no major signs of toxicity to either agent. Bleomycin was injected to a mean total dose of 292 units (range, 45-1300 units) over a mean of 28.6 weeks (range, 1-95 weeks). Seven patients had at least one significant febrile reaction within 4 hours of bleomycin administration and in three patients it was accompanied by transient hypotension. One of them had fever to 107.2°F followed

TABLE 1.—Clinical parameters and response rates in 22 patients treated with bleomycin and CCNU

| | Response | | |
|---|---|---|---|
| | Complete | Partial | None |
| No. of patients | 2 | 11 | 9 |
| Male/female | 1/1 | 6/5 | 7/2 |
| Mean age in yrs (range) | 49.5(26,73) | 42.1 (16-73) | 41.5 (17-70) |
| Mean prior duration of disease in yrs (range) | 1.25 (½,2) | 3.1 (¼-11) | 2.4 (⅜-9) |
| Histology* | 1 NS, 1 MC | 5 NS, 3 MC, 3 LD | 1 NS, 5 MC, 3 LD |
| Mean response duration in mos (range) | 26 (18,34+) | 12.2 (6-21) | — |
| Mean survival from onset of treatment in mos (range) | 27 (20,36+) | 17.5 (8-29+) | 2.2 (0-5) |

*NS = nodular sclerosing; MC = mixed cellularity; LD = lymphocyte depletion.

by profound hypotension and died 6 hours after receiving his first dose of 20 units of bleomycin given iv. The drug was considered to have played a major role in this death. Two patients had cutaneous reactions which cleared upon interrupting bleomycin briefly and did not recur with reinstitution of therapy. In addition, two patients had restrictive lung disease accompanied by impaired diffusion capacity and radiographic changes consistent with bleomycin-induced interstitial pulmonary fibrosis. One patient was a 16-year-old boy who received 165 units of bleomycin in 10 weeks. His lung disease responded to prednisone and he is alive on other therapy 29 months after bleomycin therapy was started. A 38-year-old woman died of respiratory failure after the administration of 320 units of bleomycin in 20 weeks. Permission for autopsy was denied. Bleomycin was considered contributory in her death although she was known to have had pulmonary Hodgkin's disease before being placed on the bleomycin-CCNU regimen. A 73-year-old woman and a 26-year-old man received 485 and 1300 units of bleomycin respectively without changes in serially followed chest roentgenograms, pulmonary functions, and diffusion capacities. (The latter patient will be the subject of a separate report.) No incidences of drug-related hepatic or renal toxicity were observed in this study.

CCNU was administered every 6 weeks to a mean total dose of 648 mg (range, 100-2000 mg) in a mean of three and three fourths doses (range, one to ten doses). The 22 patients received a total of 82 single doses. All patients had transient nausea within a few hours of each dose of CCNU given orally. Neutropenia and thrombocytopenia were observed (table 2) with a mean neutrophil nadir of 2600 cells/mm$^3$ at 36 days and a mean platelet count nadir of 116,000 cells/mm$^3$ at 38 days. Two instances of platelet counts < 25,000 cells/mm$^3$ and one of neutrophil count < 1000 cells/mm$^3$ were observed. However, cytopenias were transient. There were no cases of bacterial infection attributable to CCNU-induced leukopenia, and only one patient required temporary supportive care for a platelet count which dropped to 4000 cells/mm$^3$.

## DISCUSSION

Bleomycin as a single agent has been found to be effective in inducing complete remissions in 5% and partial remissions in 39% of evaluable lymphoma patients (7). Response rates in Hodgkin's disease as high as 62% have been reported but remission durations are usually brief (8). BCNU, an iv administered congener of CCNU, has been reported to yield a 47% response rate in advanced resistant Hodgkin's disease (9). Both BCNU and bleomycin are most effective during the $G_2$ phase of the cell cycle, and their use in combination has been suggested to promote increased tumor cell synchronization, as well as for the improved therapeutic index resulting from their different toxicities (10). Because of the greater ease of CCNU administration, and because of its apparent superiority over BCNU in Hodgkin's disease (11), we chose to combine CCNU with bleomycin in the treatment of MOPP-resistant stage IVB Hodgkin's disease.

The effectiveness of this combination is shown by the 72% response rate in patients surviving > 1 month. However, the low proportion of complete responses and the relatively brief duration of most of the partial responses have been distressing. Preliminary reports of experience with combinations of bleomycin with vincristine, prednisone, adriamycin, and either mechlorethamine (12) or DTIC (13) in MOPP-resistant Hodgkin's disease have appeared. The response rates are similar to ours but complete remissions are more frequent. We prefer not to re-expose patients to even nonmyelotoxic agents in the MOPP program once they have shown resistance to MOPP. Thus, since the comple-

TABLE 2.—Neutrophil and platelet count nadirs after 82 courses of CCNU

| Neutrophil nadir (cells/mm$^3$) | No. of courses | Platelet nadir (cells/mm$^3$) | No. of courses |
|---|---|---|---|
| ≥ 4000 | 19 | ≥200,000 | 20 |
| 3000-3999 | 21 | 100,000-199,000 | 31 |
| 2000-2999 | 30 | 50,000-99,000 | 22 |
| 1000-1999 | 11 | 25,000-49,000 | 7 |
| <1000 | 1 | <25,000 | 2 |
| Range: 600-4800 | | Range: 4000-300,000 | |
| Mean: 2600 | | Mean: 116,000 | |

tion of this study, we have added adriamycin and vinblastine to the bleomycin-CCNU regimen with the adriamycin, bleomycin, and vinblastine given in six monthly cycles and the CCNU in alternate cycles. Preliminary results suggest response rates comparable to those reported here, with a significantly higher complete remission rate. We are hopeful that remission durations will be longer as well.

## LIST OF COMPOUNDS

**Adriamycin:** NSC-123127; CAS reg. No. 23214-92-8

**BCNU:** NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

**Bleomycin:** NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

**CCNU:** NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

**COPP** = cyclophosphamide, vincristine, prednisone, and procarbazine

**Cyclophosphamide:** NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

**DTIC:** NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

**Mechlorethamine:** NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard

**MOPP** = mechloretnamine, vincristine, prednisone, and procarbazine

**Prednisone:** NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

**Procarbazine:** NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

**Vinblastine:** NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

**Vincristine:** NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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# Trial of Hydrazine Sulfate (NSC-150014) in Patients With Cancer[1,2,3]

**Manuel Ochoa, Jr, Robert E. Wittes, and Irwin H. Krakoff[4,5]**

Gold (1) has proposed that "the combination of anaerobic tumor energy production and reconversion of the resulting lactate (and other precursors) to glucose via gluconeogenesis in normal tissues, is [to be] considered to constitute a 'metabolic circuit' by means of which the body is depleted of its energy reserves and the process of cachexia maintained." It was further suggested that interruption of gluconeogenesis by inhibition of phosphoenolpyruvate carboxykinase could result in an anticancer effect. Inhibition of in vivo growth of four animal tumors by hydrazine sulfate was reported (2,3) and was followed by preliminary clinical trials (4). Studies to evaluate the efficacy of hydrazine sulfate as an anticancer agent in humans were undertaken at the Memorial Sloan-Kettering Cancer Center.

## METHODS

Hydrazine sulfate was supplied as an unsterile powder and was stored in a brown glass capped container maintained in a desiccator jar containing calcium sulfate. Aliquots were weighed on a torsion balance to the nearest milligram and were transferred to gelatin capsules for administration. Filler compounds were not added.

Hydrazine sulfate was given at this center to patients with disseminated nonresectable neoplasms. The drug was administered in gelatin capsules by mouth, ½ to 2 hours before a meal. Single doses were administered in midmorning and multiple doses were separated by 5 hours. Other medications were not administered from ½ hour before to 1 hour after treatment with hydrazine sulfate. With one exception, patients were given 35 mg/m$^2$ of the drug once daily for the first 4 days and twice daily on Days 5-8. The dosage was subsequently increased in selected patients after evaluation of the response and toxicity. The single exception was the first patient who was treated with an incremental regimen which began with 1 mg and progressed to 30 mg twice daily × 10 days. The lack of toxicity permitted the initiation of the protocol as outlined above.

Erythrocyte, leukocyte, platelet, reticulocyte, and differential white blood cell counts were performed three times weekly. The following measurements were done weekly: urinalysis; biochemical measurements (calcium, phosphorus, blood urea nitrogen, total protein, albumin, bilirubin, serum glutamic oxaloacetic transaminase [SGOT], lactic dehydrogenase [LDH], alkaline phosphatase, uric acid, 5′-nucleotidase, creatinine, sodium, potassium, chloride, carbon dioxide); serum protein electrophoresis; and prothrombin time. An initial chest roentgenogram, radiographic skeletal survey, liver scan, and electrocardiogram were obtained prior to therapy and were repeated as indicated by the patient status. Electromyography and electroencephalography were performed respectively with a Teca TE4 electromyograph and a Beckman TD electroencephalograph.

All patients were examined daily by one of us (Dr. Ochoa) in addition to the physician of record. All patients had malignant neoplastic diseases which could be measured by physical examination, a laboratory determination, or both. Patients were evaluated with respect to both their physical and functional status as determined by the Karnofsky performance status scale (5).

## RESULTS

Twenty-nine of 32 patients entered into the study received adequate therapy (table 1). The adequacy of treatment was defined by the appearance of a pharmacologic activity or biochemical change which could be attributed to the drug or by treatment for at least 1 week. Twenty-five patients received therapy for at least 2 weeks and 25 patients showed clinical or pharmacologic changes which were attributed to the administration of hydrazine sulfate. Three of

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[3]Hydrazine sulfate: CAS reg. No. 10034-93-2. The hydrazine sulfate used in these studies was synthesized by Eastman Kodak Co (lot 301 E3).

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the 32 patients were considered inadequately treated for evaluation.

The performance status of 18 of the patients was rated from 50% to 90%. Thus, although the malignant neoplasm was disseminated and advanced, the majority of the patients were not in a terminal state at the time of entry into the study (table 1). The histology of the neoplasms is recorded in table 1; the majority were adenocarcinomas.

The major clinical side effects were gastrointestinal and neurologic (table 2). Nausea, vomiting, and anorexia were mild. They did not preclude ingestion of food or medication and tended to occur early, generally within 2 weeks of the start of treatment. The gastrointestinal toxicity subsided within 24 hours of cessation of therapy.

The neurologic toxicity was more common, and it tended to occur late and persist longer than the gastrointestinal toxicity. The paresthesia affected both upper and lower extremities; finer motor functions (eg, writing) were impaired. The paresthesia persisted to the time of death for most patients; in two survivors the nerve deficits gradually subsided over a period of 2 months after cessation of treatment. Other neurologic changes, both central and peripheral (table 2), subsided within 1 week after treatment was terminated. Confusion was induced twice in one patient. The initial change in mentation was apparent 9 days after therapy started, subsided within 2 days of cessation of treatment, and reappeared within 4 days after reinstitution of therapy.

Minor decrease in fasting blood sugar was the most frequent laboratory abnormality (table 3). It appeared early—within 10 days in 11 of the 17 patients who manifested this alteration and within 19 days in two other patients. In eight patients the blood glucose ranged from 80 to 89 mg/100 ml, in four patients from 70 to 79 mg/100 ml, in two patients from 60 to 69 mg/100 ml, and in only one patient was it <50 mg/100 ml (43 mg/100 ml). Cessation of therapy resulted in recovery of the fasting glucose concentration. Elevations in the serum activity of alkaline phosphatase were noted in eight patients (at 2, 2, 7, 14, 31, 33, and 57 days). The magnitude of the elevation was modest, less than twice the baseline value in five of the eight patients. The highest value was 598 international units (IU)/ml. The abnormality of the alkaline phosphatase did not correlate with lowered blood sugar.

TABLE 1.—Patient characteristics

| | No. of patients |
|---|---|
| Patients entered | |
| Adequately treated | 29 |
| Inadequately treated | 3 |
| | 32 |
| Performance status (Karnofsky scale) | |
| <50% | 11 |
| 50%-90% | 18 |
| 100% | 0 |
| Diagnosis | |
| Adenocarcinoma | |
| Ovary | 5 |
| Colorectal | 4 |
| Breast | 3 |
| Lung | 2 |
| Pancreas | 2 |
| Endocervix | 1 |
| Fallopian tube | 1 |
| Prostate | 1 |
| Uterus | 1 |
| | 20 |
| Epidermoid | |
| Cervix | 1 |
| Larynx | 1 |
| | 2 |
| Miscellaneous | |
| Bladder | 1 |
| Esophagus (leiomyosarcoma) | 1 |
| Kidney (granular cell) | 1 |
| Melanoma | 3 |
| Pleura (mesothelioma) | 1 |
| | 7 |

TABLE 2.—Incidence of clinical side effects

| Type | Frequency | Time of onset (days) |
|---|---|---|
| Gastrointestinal | | |
| Nausea | 6 | 3-22 |
| Vomiting | 5 | 3-33 |
| Anorexia | 5 | 7-37 |
| Pyrosis | 3 | 2 hrs-11 days |
| Diarrhea | 2 | 1-18 |
| Constipation | 1 | 5 |
| Hunger | 1 | 14 |
| Neurologic | | |
| Paresthesia | 9 | 18-83 |
| Lethargy/somnolence | 7 | 5-83 |
| Pain | 3 | 7-18 |
| Confusion | 2 | 4-24 |
| Depression | 2 | 3-23 |
| Dysgeusia | 2 | 4-14 |
| Headache | 2 | 60-83 |
| Blurred vision | 2 | 60-83 |
| Palpebral tic | 1 | 15 |
| Slurred speech | 1 | 24 |
| Singultus | 1 | 1 |
| Pruritis | 1 | 12 |
| Vertigo | 1 | 5 |
| Miscellaneous | | |
| Diaphoresis | 2 | 29 |

TABLE 3.—Incidence of laboratory abnormalities

| Type | Frequency | Time of onset (days) |
|---|---|---|
| Chemical | | |
| Hypoglycemia | 17 | 1-33 |
| Elevated alkaline phosphatase | 8 | 7-57 |
| Hyperbilirubinemia | 2 | 32-50 |
| Elevated SGOT | 2 | 5-44 |
| Elevated LDH | 2 | 13-29 |
| Neurologic | | |
| Electromyographic | | |
| Sensorimotor | 4 | — |
| Sensory | 1 | — |
| Electroencephalographic | | |
| Metabolic encephalopathy | 1 | — |
| Diffuse | 1 | — |
| Local | 1 | — |

Elevations in the serum concentrations of bilirubin (to 3.0 and 5.3 mg/100 ml), SGOT (to 107 and 171 IU/ml), and LDH (to 325 and 351 IU/ml) were seen in six patients.

Since all patients had disseminated cancer, the abnormal liver function tests cannot be definitely attributed to an effect of hydrazine sulfate.

Abnormal electromyograms correlated with peripheral neuropathies when those tests were done (table 3). Electroencephalographic changes were recorded in three patients but the interpretation of the observations is questionable because pretreatment electroencephalograms were not done.

No significant changes were observed in other parameters.

Significant subjective or objective responses were *not* observed in any of the 29 adequately treated patients. Four patients reported enhancement of their appetites from 3 to 7 days after the start of therapy. The improvement of appetites persisted for 5, 6, 7, and 14 days in these four patients but none gained weight. One patient with prostate cancer experienced a decrease in bone pain 3 days after therapy started. The modest relief of pain lasted approximately 3 weeks; the serum acid phosphatase activity increased during this period. The rate of accumulation of ascites decreased in one patient but prior paracenteses had been performed. Finally, a weight gain of 0.6 kg was experienced by a single patient but because his home environment was one of deprivation, the gain in weight might have been due to improved nutrition when hospitalized.

## COMMENT

Significant subjective improvements or objective antitumor responses were *not* observed in any of 29 patients adequately treated with hydrazine sulfate. In contrast, significant toxicity (neurologic impairment in particular) was evident.

Those antitumor compounds which are useful in the treatment of cancer in man, inhibit the biosynthesis, biophysical state, or function of DNA, RNA, or protein, or may influence cell-membrane receptors. Inhibition of gluconeogenesis was proposed by Gold as a new mechanism of antitumor effect. Gold (2) also reported the inhibition of the Walker 256 carcinosarcoma in rats by administration of hydrazine sulfate.

Subsequent studies (3) by Gold extended the observations to other solid animal tumors with similar levels of activity. In contrast, Tarnowski and Stock (personal communication) failed to detect antitumor activity by hydrazine sulfate in the following transplanted tumors: Sarcoma 180, Meth A sarcoma (ascites), B16 melanoma, and Ridgway osteogenic sarcoma. Borderline results were observed in the Walker 256 carcinosarcoma.

Gold (4) has reported, in abstract form, a collation of the responses of ten patients with advanced cancer to treatment with hydrazine sulfate of which six were said to be subjectively and/or objectively improved. The details of the design of the study, eg, collaborating investigators and institutions and total number of patients entered, were not specified. We did not observe any objective response in our patients, a group which included a wide variety of tumor types. Four of our patients had briefly improved appetites without gain of weight and one experienced a transitory decrease in bone pain without parallel improvement in the roentgenograms of the bones but with continuing elevations of the serum acid phosphatase. These infrequent, minor, transient, and subjective changes were those commonly seen with the start of any new therapy and are not considered to represent useful responses.

Major neurologic toxicity was observed in half of the patients. Gold (4) reported that "side effects were minimal" but reported torpor in two of ten patients. Hydrazine sulfate is known to produce a spectrum of effects (6) in animal systems which includes excitement, lethargy, convulsions, hypotension, arrhythmias, fatty changes in the liver, hypoglycemia, weight loss, and terminal elevations in SGOT activity. Our clinical observations with respect to toxicity (which was manifest at low doses as well as the higher ones) are qualitatively consistent with those observed in rats and rhesus monkeys, although markedly less at the doses used. Hydrazine sulfate has also been reported to be carcinogenic in animal systems (7,8).

The clinical observations recorded in this report fail to support a role for hydrazine sulfate as an

antitumor agent we conclude that its clinical utilization is not warranted at present and do not plan further trials.

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# Evaluation of CCNU (NSC-79037) Used for the Prevention of CNS Involvement in Burkitt's Lymphoma[1,2,3]

**Brief Reports and Preliminary Communications**

**John L. Ziegler,[4,5] Ian T. Magrath,[4] Francis K. Nkrumah,[6] Ida Virginia Perkins,[6] and Richard Simon[7,8]**

Approximately one third of the patients with Burkitt's lymphoma develop meningeal involvement (malignant pleocytosis) as a manifestation of relapse (1,2). The present study was designed to evaluate whether CCNU given in addition to treatment with cyclophosphamide[9] would have any significant prophylactic value against the development of CNS involvement in Burkitt's lymphoma in a randomized trial. This study was performed collaboratively between the Lymphoma Treatment Center (Uganda Cancer Institute, Makerere University Medical School, Kampala) and the Burkitt's Tumor Project (University of Ghana Medical School, Accra).

## PATIENTS AND METHODS

Previously untreated patients with Burkitt's lymphoma were staged clinically according to criteria previously described (3). For all patients cerebrospinal fluid (CSF) was examined on admission and serially for 1 year. Patients who had no malignant cells in the CSF prior to chemotherapy received initial treatment with intravenous (iv) cyclophosphamide (40 mg/kg). Two weeks later they were randomized by stage to receive *either* a second iv 40-mg/kg dose of cyclophosphamide alone *or* a second iv 40-mg/kg dose of cyclophosphamide plus 70 mg/m$^2$ of CCNU given orally. For patients in the Ghana trial treatment was stopped after the attainment of a complete remission. Patients in the Uganda trial were subsequently randomized (stratified by stage and by receipt of CCNU) to receive or not to receive a 10-week trial of bacillus Calmette-Guérin (BCG) immunotherapy (4).

## RESULTS

A total of 65 evaluable patients were entered into the trial between July 1970 and July 1972; 24 were treated in Ghana and 41 were treated in Uganda. The data were analyzed separately and the results were found to be comparable; therefore, for the purposes of this analysis the data are combined from both centers. In the Uganda trial, it was further noted that BCG immunotherapy had no effect on the pattern or frequency of relapse (4).

Thirty-two patients received CCNU and 33 patients were controls; no distribution differences with respect to initial stage, response to therapy, BCG immunotherapy, age, or sex were noted. All but four patients (two in each group) achieved complete responses and were evaluable for analysis of remission duration and site of relapse.

No significant difference in CNS relapse rate or remission duration was noted between the group receiving CCNU (nine CNS relapses) and the controls (11 CNS relapses). Patients with disseminated (stages III or IV) tumor were at greater risk of developing CNS relapse. There was no difference in the overall relapse rate (systemic relapse with or without CNS involvement) with respect to CCNU or BCG treatment (4). CNS relapse (with or without neuropathy) was accompanied or preceded by relapse in other sites in five of nine patients in the CCNU group and seven of 11 controls. Finally, no differences in myelotoxicity were observed in the CCNU group compared to the controls.

[1]Received Mar 20, 1975; revised July 29, 1975; accepted Aug 27, 1975.

[2]The Uganda Cancer Institute is supported by contract PH43-67-1343 from the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health, Education, and Welfare (DHEW). The Burkitt's Tumor Project is supported by the Division of Cancer Cause and Prevention, NCI, NIH, DHEW.

[3]CCNU: CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-. All drugs used in this study were supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment (DCT), NCI.

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[8]We are grateful to Dr. E. C. Christian (Department of Pathology, University of Ghana Medical School) and Dr. A. C. Templeton (Department of Pathology, Makerere University Medical School) for reviewing all histologic and cytologic material.

[9]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,-3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Analysis of survival of CCNU-treated patients and controls revealed an unexpected and statistically significant increase in mortality in the CCNU group as shown in figure 1 ($P<0.005$ by Mantel-Hanszel actuarial chi$^2$). This increase could not be explained by differences in age, reductive surgery, treatment response, subsequent BCG immunotherapy, myelotoxicity from treatment (drug-related death), or an imbalance in initial clinical stage. In the majority of patients in the CCNU-treated group death resulted from progressive growth of drug-resistant tumors.

## DISCUSSION

In the present trial there was no evidence that CCNU prevented or delayed relapse involving the CNS, as preliminary findings had indicated. This failure may have been a result of: (*a*) inadequate dosage; (*b*) insensitivity of tumor cells to the drug; or (*c*) spread to the CNS from residual systemic tumor occurring after CCNU had been metabolized or eliminated by the patient. Since the dose of CCNU used in this trial was half that usually administered when the agent is used alone, it is possible that insufficient concentration of drug reached the tumor. The nitrosoureas have demonstrated activity in Burkitt's lymphoma, but they are not considered first-line agents. Finally, the pathogenesis of neurologic involvement in Burkitt's lymphoma is not known, and access to the CNS by residual systemic tumor may be a continuing process.

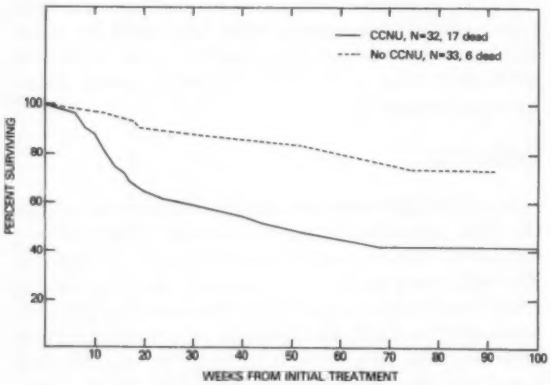


FIGURE 1.—Survival of CCNU-treated patients and controls with Burkitt's lymphoma.

The increased mortality of patients in the CCNU arm of the present trial bears additional comment. We can offer no explanation for this, particularly in view of the absence of toxicity. The major cause of death in this group of patients was drug-resistant tumor. This observation may be relevant to the treatment of other tumors with CCNU in combination with cyclophosphamide, and it would appear to be important to determine whether such a phenomenon is the result of a perturbation of cellular repair mechanisms by CCNU.

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# High-Dose Cyclophosphamide (NSC-26271) for Recurrent or Progressive Ovarian Adenocarcinoma[1,2]

**Brief Reports and Preliminary Communications**

**M. Steven Piver,[3] Joseph J. Barlow, and Whan S. Chung[4]**

Approximately 50% of the women who have metastatic ovarian adenocarcinoma will respond to primary therapy with alkylating agent chemotherapy (1,2). A recent report by Buckner et al showed a clinical response rate of 89% (eight of nine patients) in patients treated with high-dose cyclophosphamide (120 mg/kg) as primary treatment for metastatic ovarian adenocarcinoma (3). In addition, two thirds of these patients showed a complete response to this therapy. Although the number of patients receiving high-dose cyclophosphamide was small, a very high total response rate and a high complete response rate suggest that there may be a dose-response curve with the use of increasing doses of cyclophosphamide. In women no longer responding to alkylating agent chemotherapy, subsequent forms of chemotherapy have shown little effectiveness. Because of the excellent response rate to high-dose cyclophosphamide as primary therapy, an evaluation of such therapy in women with recurrent or progressive ovarian adenocarcinoma was undertaken.

## MATERIALS AND METHODS

Eight women with recurrent ovarian adenocarcinoma were treated with high-dose cyclophosphamide (70-100 mg/kg) over a period of 2-7 days. Histopathology consisted of serous adenocarcinoma (4), mucinous adenocarcinoma (3), and clear cell adenocarcinoma (1). Six patients were stage III (abdominal metastases) and two were stage IV by virtue of pleural effusion. Six patients had previously been treated with alkylating agent chemotherapy alone or in combination with 5-fluorouracil (5-FU) and actinomycin D. Three patients had shown complete response to melphalan and three showed no response to alkylating agent chemotherapy. One patient had recurrent ovarian adenocarcinoma after 5-FU and one after pelvic irradiation. Of the six patients previously treated with alkylating agent chemotherapy primarily, three subsequently received low-dose cyclophosphamide (25-30 mg/kg) with actinomycin D and 5-FU or methotrexate and had progressed on this therapy.

Intradermal skin tests consisted of tuberculin (intermediate purified protein derivative, Connaught, 0.1 cc); Varidase (100 units of streptokinase/1 ml); *Monilia (Candida albicans* 1:1000); mumps (Lilly); and keyhole-limpet hemocyanin (1 mg/ml). Skin tests were graded at 48 hours: anergic = 0-4 mm of induration; reactive = > 5 mm of induration. Five patients received one course, two patients received two courses, and one patient received three courses of high-dose cyclophosphamide. The dose of cyclophosphamide was reduced from 120 mg/kg as reported by Buckner et al (3) to 70-100 mg/kg because of the patients' previous therapy. In addition, they received fewer courses of therapy because of the severe toxicity sustained.

## RESULTS

### Hematologic Toxicity

Six of the eight patients had severe leukopenia of < 400 cells/mm$^3$ (range, 44-400 cells) after the first course of high-dose cyclophosphamide. The leukopenic nadir occurred on Day 8 (range, Days 2-10). The only patient receiving three courses of therapy had severe leukopenia of 66 cells/mm$^3$ after the first course (70 mg/kg) and 70 cells/mm$^3$ after the third course (60 mg/kg). Only one patient's white blood cell count did not decrease to < 1000 cells/mm$^3$. Six of the eight women had no significant thrombocytopenia but two showed severe thrombocytopenia of 5000 and 22,000 cells/mm$^3$ respectively, both after the first course of therapy.

### Septicemia

Five of the eight patients developed septicemia, all at the time of profound leukopenia. Septicemia occurred in four patients after the first course and in one patient after the third course of chemotherapy. The patients were successfully treated with antibiotic therapy consisting primarily of high-dose Keflin and carbenicillin.

[1]Received Apr 15, 1975; revised Aug 1, 1975; accepted Aug 20, 1975.

[2]Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

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[4]Department of Gynecology, Roswell Park Memorial Institute, Buffalo, NY.

### Antitumor Response

No tumor response was shown in seven of the patients. One of the patients had a partial response (control of effusion and 50% decrease in abdominal mass), but this lasted only 1 month.

### Skin Test

Six of the eight patients were anergic to the five skin tests, including the one patient who showed a short partial tumor response. Two patients had reactions to skin tests, but did not respond to chemotherapy.

## DISCUSSION

Response to chemotherapy in women no longer responding to initial alkylating agent chemotherapy for metastatic ovarian adenocarcinoma has been poor. However, Smith and Rutledge did report a response rate of 39% with actinomycin D, 5-FU, and cyclophosphamide in 47 patients no longer responding to melphalan (2). We repeated the same regimen in 11 consecutive patients who were no longer responding to melphalan and had no responses (4). Because of the excellent clinical response rate of 89% using high-dose cyclophosphamide as primary therapy for stage III ovarian adenocarcinoma reported by Buckner et al, a study was undertaken to use high-dose cyclophosphamide for recurrent ovarian adenocarcinoma. Eight women were treated with high-dose cyclophosphamide (70-100 mg/kg per course) for recurrent ovarian adenocarcinoma. Only one patient had a partial response (12%) and this lasted only 1 month. In addition, even at this dose level which was less than that reported by Buckner, severe leukopenia developed in six of the eight women (< 400 cells/mm$^3$) after the first course of therapy. At the time of severe leukopenia five patients (62%) had septicemia.

In using high-dose cyclophosphamide as primary therapy for advanced ovarian adenocarcinoma, Young et al (5) reported no superiority for this regimen as compared to oral melphalan. However, there was significant toxicity using the intensive cyclophosphamide therapy.

The reason for the poor response to second-line chemotherapy in ovarian adenocarcinoma is unknown. One theoretical possibility for the poor response in these eight women is that the tumor masses were large and, therefore, had partially outgrown their blood supply. Thus, sufficient quantities of the chemotherapeutic agents may not have reached the tumor. Also, it is possible that the larger the tumor the larger the number of blocking antibodies. In addition, the depressed cellular immunity (as shown by anergy in six of the eight patients who failed to respond to intradermal skin tests prior to high-dose cyclophosphamide) could account for the poor response to chemotherapy. However, since six of the eight patients had previously been treated with alkylating agents, intrinsic resistance to the alkylating agents must be considered. Theoretical possibilities for improving the above three situations include reductive tumor surgery, where possible, to reduce the tumor load, to improve the blood supply to the tumor, and possibly to reduce the number of blocking antibodies. In addition, those patients who are anergic prior to second-line chemotherapy could be treated with nonspecific immunotherapy in an effort to improve their immune system and thus possibly improve their response to subsequent chemotherapy.

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# Combination Vincristine (NSC-67574) and Hydroxyurea (NSC-32065) for Metastatic Renal Carcinoma[1,2]

**Brief Reports and Preliminary Communications**

**Douglas E. Johnson,[3] Luis Rodriguez, Paul Y. Holoye, and Melvin L. Samuels[4]**

Metastatic renal cell carcinoma is one of the many solid tumors that remains very resistant to chemotherapy (1). Hydroxyurea has been reported to show activity (2-5) although the responses are partial and brief. Recently, Pouillart et al (6) have advocated the sequential combination of a drug that is a mitotic spindle poison, such as vincristine, followed by a second drug with different action but with some known activity against the specific tumor under study. We have been interested in using periwinkle alkaloids in this manner and have shown a high complete response rate with probable chemotherapy cures when a large dose of vinblastine is followed by bleomycin in the treatment of testicular neoplasia (7). A similar approach using vincristine followed by hydroxyurea seemed worthy of exploratory trials in renal cell carcinoma.

## MATERIALS AND METHODS

Fifteen unselected patients with metastatic renal cell carcinoma were entered in this study between July 1, 1974, and December 31, 1974. The two requirements for admission into the study were (*a*) the leukocyte and platelet counts had to be >5000 and >150,000 cells/mm$^3$ respectively; and (*b*) there had to be measurable metastatic disease. The symptom performance status was rated as follows: 0 = no symptoms (working fulltime); 1 = symptoms (fully ambulatory but unable to work fulltime); 2 = confined to bed <50% of the time; 3 = confined to bed >50% of the time; and 4 = bedridden. The patient population is summarized in table 1. The mean symptom status of 1.5 indicated most of the patients were ambulatory. Objective tumor response was determined by serial measurements. The grading of response was as follows: (*a*) complete (all measurable tumor disappeared); (*b*) partial (a 50% decrease in the product of the maximum diameter and the perpendicular maximum diameter of all measurable lesions); (*c*) mixed (one or more tumor masses showed a partial response while other measurable disease remained unchanged or showed less than a partial response); (*d*) stable (no change in any of the measurable lesions and no evidence of new lesions); and (*e*) no response (continued growth of measurable disease).

Leukopenia was classified as minimal (2000-2900 cells/mm$^3$), moderate (1000-1900 cells/mm$^3$), or severe (< 1000 cells/mm$^3$). Thrombocytopenia was classified as minimal (100,000-125,000 cells/mm$^3$), moderate (50,000-99,000 cells/mm$^3$), and severe (<50,000 cells/mm$^3$).

All patients received vincristine at doses of 1 mg/m$^2$ iv on Days 1 and 2 followed by hydroxyurea at a dose of 2 g/m$^2$ in four divided doses for 5 days (Days 3-8). If the dose of hydroxyurea was tolerated without any fall in the leukocyte or platelet count it was increased by an increment of 25% in each subsequent course. The treatment program was repeated at 21-day intervals for a minimum of three courses. Most therapy was given on an outpatient basis.

## RESPONSE AND TOXICITY

Table 1 summarizes the clinical data on each patient. There were no partial or complete responses. In two patients, metastatic disease remained stable while the patients were receiving the drug program. In one of these patients, drugs had to be discontinued after 5 months due to severe peripheral neuropathy. In two other patients, partial regression of pulmonary metastases was shown, but development of bone and subcutaneous metastases occurred at other sites.

Toxic manifestations are summarized in table 2. All patients developed evidence of drug toxicity some time during their course of therapy. Leukopenia occurred in 11 patients during therapy, and in 45 evaluable courses it was minimal in 15, moderate in ten, and severe in seven instances. Thrombocytopenia occurred in three patients and was moderate in one and severe in two patients. There was one drug death from presumed infection secondary to severe myelosuppression after the third course of therapy.

[1]Received May 7, 1975; revised Aug 4, 1975; accepted Aug 20, 1975.

[2]Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1). Hydroxyurea: CAS reg. No. 127-07-1.

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[4]The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston.

TABLE 1.—Clinical summary

| Patient age (yrs) | Sex | Original tumor site | Symptom status* | Total dose of hydroxyurea (g) | No. of courses | Response | Survival in wks since— | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Diagnosis | Chemotherapy |
| 67 | M | Lungs | 1 | 60 | 3 | Stable | 20 | 20 (A)† |
| 49 | M | Lungs | 1 | 160 | 5 | Stable | 16 | 12 (A) |
| 48 | M | Lungs | 1 | 48 | 3 | Progression | 16 | 16 (D) |
| 57 | M | Lungs | 1 | 185 | 7 | Progression | 24 | 16 (A) |
| 59 | F | Lungs | 1 | 81 | 4 | Progression | 16 | 12 (D) |
| 55 | F | Lungs | 1 | 186 | 8 | Progression | 88 | 28 (A) |
| 52 | F | Lungs | 1 | 60 | 3 | Progression | 60 | 12 (A) |
| 73 | M | Lungs | 1 | 60 | 3 | Progression | 12 | 10 (A) |
| 53 | M | Liver | 1 | 42 | 3 | Progression | 16 | 12 (D) |
| 49 | M | Brain/lungs | 3 | 173 | 7 | Progression | 36 | 20 (A) |
| 50 | M | Bone | 1 | 103 | 4 | Progression | 24 | 12 (D) |
| 42 | F | Bone | 2 | 123 | 4 | Progression | 172 | 10 (A) |
| 73 | M | Bone/lungs | 2 | 133 | 6 | Progression | 56 | 12 (A) |
| 56 | F | Bone/lungs | 3 | 75 | 4 | Progression | 12 | 12 (D) |
| 77 | M | Bone/lung | 2 | 61 | 3 | Progression | 16 | 16 (D) |

*See text.
†A = alive; D = dead.

## DISCUSSION

The selection of vincristine instead of vinblastine may be criticized on the grounds that renal cell carcinoma is essentially nonresponsive to vincristine (8) whereas in 60 patients studied 14 objective responses to vinblastine have been reported (2,9). However, both vinblastine and hydroxyurea are myelosuppressive and we felt it would be difficult to combine them in meaningful doses, especially for outpatient use.

Continuous iv administration of hydroxyurea is desirable since the peak blood level after a single oral dose occurs in 2.5 hours with very little detectable drug after 5.5 hours (10). However, in our study the drug was given orally every 6 hours around the clock for 5 days because of the short supply of the iv preparation. Since myelosuppression appeared in the majority of patients, the dose was considered to have been adequate.

TABLE 2.—Summary of toxic manifestations

| Toxic manifestations | No. of patients |
|---|---|
| Hematologic | |
| Leukopenia (<3000 cells/mm³) | 11 |
| Thrombocytopenia (<125,000 cells/mm³) | 3 |
| Anemia (<10 g/100 ml) | 10 |
| Gastrointestinal | |
| Nausea and vomiting | 5 |
| Diarrhea | 1 |
| Dermatologic | |
| Alopecia | 15 |
| Maculopapular rash | 2 |
| Neurologic | |
| Peripheral neuropathy | 6 |
| Paresthesias | 6 |
| Hoarseness | 2 |

Previous investigators have attempted to correlate dose with response. Five of 14 patients with renal cell carcinoma who received >50 g of hydroxyurea showed objective responses lasting 2-5 months in a study by Nevinny and Hall (4). Thirteen of our 15 patients received a dose of 60 g or more of hydroxyurea with only stabilization of disease shown in two patients. The therapeutic value of this combination drug program in the treatment of advanced renal carcinoma appears negligible.

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# Methyl-CCNU (NSC-95441) Versus Methyl-CCNU Plus Cyclophosphamide (NSC-26271) in Advanced Gastrointestinal Cancer[1,2,3]

**Brief Reports and Preliminary Communications**

**Anna Rossi, Antonio Riva, and Gianni Bonadonna[4,5]**

Studies by Mayo et al (1) in Lewis lung carcinoma have suggested a synergism between methyl-CCNU and cyclophosphamide. Since, in advanced gastrointestinal cancer, both methyl-CCNU (2) and high intermittent doses of cyclophosphamide (3) were reported to produce objective responses, a controlled study was started at our Institute to test the synergistic effect of methyl-CCNU plus cyclophosphamide in the treatment of this disease.

## Patients and Methods

A total of 62 ambulatory patients with histologically proven diagnoses of advanced adenocarcinoma of the stomach or large intestine were entered into the study. Each patient had one or more measurable areas of evaluable disease which were proven, whenever possible, through biopsy. None of the patients had a performance status below 50. The life expectancy of each patient before the start of treatment was not less than 6 weeks.

The patients were randomly allocated to treatment with either methyl-CCNU (33 patients) or methyl-CCNU plus cyclophosphamide (29 patients). Patients were stratified according to primary site of tumor (stomach or colorectal), status of primary tumor (resected or unresected), and site of dominant lesion (hepatic, pulmonary, or other).

When administered alone, methyl-CCNU was given orally at an initial dose of 200 mg/m$^2$ and cycles were repeated in the absence of myelosuppression at 6-week intervals. In combination with cyclophosphamide, the dose of methyl-CCNU was 120 mg/m$^2$ every 6 weeks. Cyclophosphamide was administered iv every 3 weeks at a dose level of 600 mg/m$^2$. An initial lower dose was planned in patients older than 65 years (methyl-CCNU, 170 mg/m$^2$ as a single agent; cyclophosphamide, 400 mg/m$^2$ in combined therapy).

The categories of response employed in this study were as follows: complete remission was defined as the complete disappearance of all signs of measurable disease with a return to normal of biochemical tests for a minimum of 1 month; partial remission was categorized as a decrease by at least 50% in the product of the largest perpendicular diameters of all measurable areas of known cancer; objective improvement was defined as a decrease of 25%-50% of all measurable lesions.

Evaluable patients (methyl-CCNU, 24 patients; methyl-CCNU plus cyclophosphamide, 17 patients) were considered to be only those who received a minimum of two cycles of therapy; 58% and 59%, respectively, had received no prior therapy. In both arms of the study, all patients who had previously received chemotherapy were given 5-fluorouracil.[6]

The comparison of response is reported in table 1. Only one patient receiving the combination achieved a complete response. She had a cutaneous metastasis as the sole site of known disease. No statistical difference between the two groups was evident in terms of response rate and duration of response. Also, no meaningful response difference was observed in relation to the site of primary tumor (stomach, one of five [single agent] vs none of three [combination]; colorectal, one of 19 vs three of 14). The median survival time was longer in both treatment groups in responsive patients as compared to nonresponsive patients (methyl-CCNU, 14 vs 6.5 months; methyl-CCNU plus cyclophosphamide, 15.5 vs 6.5 months). Once more, however, no difference exists between the two treatment groups.

Table 2 reports the toxic manifestations. Early toxicity was exclusively represented by transient nausea and vomiting which occurred within a few hours after drug administration. Delayed myelosuppression was the most prominent, although reversible, side effect. Cumulative marrow toxicity, when present, never caused the discontinuation of treatment.

[1]Received June 2, 1975; revised Aug 12, 1975; accepted Aug 20, 1975.

[2]Supported in part by contract NO1-CM-33714 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Methyl-CCNU: CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[4]Istituto Nazionale Tumori, Milan, Italy.

[5]*Reprint requests to:* Dr. Gianni Bonadonna, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, Milano 20133, Italy.

[6]5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

TABLE 1.—Comparison of response

| Type of response* | Methyl-CCNU (24 patients) | | Methyl-CCNU + cyclophosphamide (17 patients) | |
|---|---|---|---|---|
| | No. of patients | Duration of response in mos (range) | No. of patients | Duration of response in mos (range) |
| No response | 18 | | 13 | |
| Objective improvement | 4 | 4(3-4.5) | 1 | 8 |
| PR | 2 | 8.5(7.5-9.5) | 2 | 5(2-8.5) |
| CR | 0 | | 1 | 5 |
| CR + PR | 2(8%) | | 3(17%) | |
| Total No. responding | 6(25%) | | 4(23%) | |

*See Patients and Materials for definitions.

TABLE 2.—Frequency of toxic manifestations

| Toxic effect | % Patients receiving— Methyl-CCNU | Methyl-CCNU + cyclophosphamide |
|---|---|---|
| Nausea and vomiting | 66 | 75 |
| Anemia* | 29 | 23 |
| Leukopenia | | |
| 3999-2000 cells/mm³ | 18 | 45 |
| <1999 cells/mm³ | 18 | 0 |
| Thrombocytopenia | | |
| 129,000-80,000 platelets/mm³ | 11 | 45 |
| <79,000 platelets/mm³ | | 8 |

*Decrease in hemoglobin value >2 g below initial values without evidence of blood loss.

The average percent of the projected dose administered during the first six cycles was 82% in the group receiving methyl-CCNU alone. In the group receiving combination chemotherapy, the percent administered was 88% for methyl-CCNU and 76% for cyclophosphamide.

## Comment

The results of this controlled study, while confirming the limited activity of methyl-CCNU, failed to show evidence of any synergistic therapeutic effect of methyl-CCNU plus cyclophosphamide over methyl-CCNU alone in patients with advanced gastrointestinal cancer. Our results, however, in far advanced human neoplasia cannot exclude a synergistic activity between methyl-CCNU and cyclophosphamide in the presence of minimal residual disease, as observed in the experimental model.

In fact, in the study of Mayo et al (1), the combination was most effective in producing complete regressions and "cures" in early Lewis lung carcinoma or in the presence of metastatic disease when the primary tumor had been surgically removed. At the present moment, however, the opportunity for a trial with methyl-CCNU plus cyclophosphamide as adjuvant therapy should be weighed against the results (40%-50% response rate) which are being obtained in advanced colorectal cancer.[7]

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[7]Moertel CG. Personal communication.

# Adriamycin (NSC-123127) Plus 5-Fluorouracil (NSC-19893): A Phase I Study[1,2,3,4]

B. L. Tranum, R. L. Stephens, D. E. Lehane, B. Hoogstraten, M. Lane, and A. Haut[5,6]



Two new drug regimens capable of producing high response rates in breast cancer have been reported in the recent literature (1, 2). The five-drug regimen originally introduced by Cooper (cyclophosphamide, vincristine, methotrexate, 5-fluorouracil [5-FU], and prednisone) (1) has been studied by several investigators and was shown to give a response rate between 55% and 88% (2-4), while Hoogstraten et al (2) have shown that adriamycin alone results in a 40% remission rate. Combining one or more agents from the five-drug combination with adriamycin may reasonably be expected to add to the response rate obtained by adriamycin alone.

This study, conducted in three member institutions of the Southwest Oncology Group (SWOG), was designed to determine the optimum dose of 5-FU and adriamycin in combination.

## METHODS AND MATERIALS

Adult patients with advanced solid tumors who had not previously received adriamycin or 5-FU, had no prior history of cardiovascular disease, and had adequate hepatic function were eligible for the study. Thirty-eight of 61 patients had previously been treated with chemotherapy and radiotherapy. Prestudy evaluation included complete blood cell counts, liver and renal function tests, electrocardiograms, and chest and other roentgenograms as warranted by the extent of the disease.

The dose levels tested and the schema for drug administration are outlined in table 1. Treatment was started at the lowest dose level initially, and after determining that no undue toxicity developed, subsequent patients were treated at the next highest dose level. After receiving one complete course of therapy without appreciable toxicity, patients could be escalated to a higher dose level. Forty-four patients did not have their doses changed while receiving therapy, whereas 17 patients were treated at two or more dose levels. Adriamycin was administered iv every 3 weeks and 5-FU was given iv weekly. Adriamycin was discontinued at a total cumulative dose of 500 mg/m$^2$. Patients were taken off treatment if there was progression of disease. Drug toxicity was graded from 0 to 4 (table 2).

Before starting therapy all patients were informed as to the investigational nature of this protocol and all gave their informed consent to participate in the study.

The response to therapy was classified as complete regression, partial regression, or failure, using the strict criteria of the SWOG (5).

## RESULTS

Sixty-three patients were entered in the study. Nine patients were not evaluable for toxicity and two were not evaluable for response.

The relationship between dose level and the decision to interrupt or delay a cycle of therapy due to toxicity is noted in table 1. In no case was it necessary to delay starting the next course of therapy more than 14 days. The explanation for the high frequency of dose interruption at dose level 4 is not obvious, but 50% of the patients had altered liver function which may have exaggerated adriamycin toxicity.

Major hematologic toxicity for all 54 patients is shown in table 2. Severe or life-threatening thrombocytopenia (grade 3 or 4) was much less common than the comparable degree of leukopenia. There was no evidence of cumulative toxicity. The median day of the white blood cell nadir was 15 days after the start of therapy with a range of from 8 to 21 days.

There was no definite incidence of cardiac toxicity. Although there were cases in which sudden death

[1]Received June 4, 1975; revised Aug 1, 1975; accepted Aug 20, 1975.

[2]This study was done by the Southwest Oncology Group (Barth Hoogstraten, chairman) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following institutions participated in the study: University of Arkansas Medical Center (*B. L. Tranum* and *A. Haut*, CA-03400); Baylor College of Medicine (*M. Lane* and *D. E. Lehane*, CA-03392); University of Kansas Medical Center (*R. L. Stephens* [CA-12644] and *B. Hoogstraten* [CA-16943 and CA-12014]).

[3]Adriamycin: CAS reg. No. 23214-92-8.

5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU.

[4]Presented in part at the meeting of the American Society of Clinical Oncology, May 1975, San Diego, Calif.

[5]Appreciation is expressed to Ms. M. Gregory for preparation of the manuscript.

[6]*Reprint requests to:* Cherri Stadalman, Group Administrative Assistant, Southwest Oncology Group, 3500 Rainbow Blvd, Suite 201, Kansas City, Kan 66103.

TABLE 1.—Dose level, response, and frequency of interruption due to toxicity

| Dose level | Dose of adriamycin/ 5-FU* ($mg/m^2$) | No. of evaluable patients | % of patients— | |
|---|---|---|---|---|
| | | | Responding | With treatment interruption |
| 1 | 30/300 | 9 | 44 | 0 |
| 2 | 40/300 | 10 | 33 | 10 |
| 3 | 50/300 | 12 | 8 | 33 |
| 4 | 60/300 | 9 | 0 | 67 |
| 5 | 60/400 | 9 | 67 | 33 |
| 6 | 60/500 | 6 | 17 | 60 |
| 7 | 60/600 | 6 | 33 | 67 |
| Total | | 61 | 28 | 35 |

*Adriamycin given q3wks iv; 5-FU given weekly iv.

TABLE 2.—Hematologic toxicity

| Dose level | Treated | No. of patients— With wbc count grade—* | | | | | With platelet count grade—* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| 1 | 7 | 6 | | 1 | | | 6 | | 1 | | |
| 2 | 10 | 6 | 3 | 1 | | | 10 | | | | |
| 3 | 12 | 5 | 1 | 3 | 3 | | 10 | 1 | | | 1 |
| 4 | 6 | | | 2 | 2 | 2 | 3 | | 1 | | |
| 5 | 8 | 3 | 1 | 2 | 1 | 1 | 6 | 1 | | 1 | 2 |
| 6 | 5 | | 1 | 3 | | 1 | 5 | | | | |
| 7 | 6 | | | 1 | 1 | 4 | 4 | 1 | | 1 | |

*Toxicity criteria:

| *Grade* | *Wbc/$\mu l \times 10^{-3}$* | *Platelets/$\mu l \times 10^{-3}$* |
|---|---|---|
| 0 | > 4 | > 100 |
| 1 | 3.0-3.9 | 75-99 |
| 2 | 2.0-2.9 | 50-74 |
| 3 | 1.0-1.9 | 25-49 |
| 4 | < 1.0 | <2.5 |

occurred these deaths were attributable to progressive tumor growth. Only one patient received 500 $mg/m^2$ of adriamycin.

There was gastrointestinal toxicity at all dose levels that did not appear to be dose related. Stomatitis of various degrees was noted in 26% of the patients but was severe in only 11%. Nausea, vomiting, or diarrhea occurred in 45% of patients but was judged to be severe in only 13%. Alopecia of various degrees was universal.

Table 1 outlines the frequency of response to therapy at each dose level. Responses occurred at all levels and were no more frequent at the higher dose levels. No attempt was made to randomize the more sensitive tumor types (ie, breast cancer) among the various dose levels; therefore this finding is of limited value. Of the 61 patients evaluable for response to therapy remissions occurred in 17. Responses occurred in carcinoma of breast, lung, head and neck, rectum, and esophagus. Since one of the pri-

mary objectives of this study was to develop a better protocol for breast cancer, the results of the 17 patients with this particular tumor were analyzed separately. There were seven partial remissions, two complete remissions, and eight nonresponders. The overall response rate for breast cancer was 53%.

## DISCUSSION

It has been established that the average therapeutic dose for adriamycin alone is 60-75 mg/m$^2$ to be given iv at 3-week intervals (6,7). The toxicity has consisted mainly of leukopenia, nausea and vomiting, ulceration of mucosal surfaces, and, at doses >550 mg/m$^2$, cardiotoxicity (6, 8). O'Bryan et al (5,7) have presented data on the relationship of tumor response to dose level and concluded that 60 mg/m$^2$ was the best dose.

The toxic effects of the combined drug regimen used in this study are similar to those noted with adriamycin used alone. The Day 15 granulocytopenic nadir and its prompt recovery were also described by others in phase I studies with adriamycin alone (6).

The adriamycin and 5-FU combination is a well-tolerated regimen in which the near-full therapeutic dose of adriamycin and half the effective 5-FU dose (9) can be administered without undue toxicity. Adriamycin at a dose of 50 mg/m$^2$ administered every 3 weeks and 5-FU at a dose of 300 mg/m$^2$ administered weekly is the recommended starting dosage for patients with adequate liver function. Starting at this dose level should offer some protection against the high degree of toxicity noted at the next highest dose level. A number of patients will tolerate an escalation in the dosage to 60 mg/m$^2$ of adriamycin plus 400 mg/m$^2$ of 5-FU weekly.

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# Combination Chemotherapy With 5-Fluorouracil (NSC-19893), Methotrexate (NSC-740), and Prednisolone (NSC-9900) (FAP Protocol) for Hepatoma[1,2,3]

**Brief Reports and Preliminary Communications**

**Theera Umsawasdi,[4] Termchai Chainuvati, Sawadh Hitannant, Prasert Nilprabhassorn, and Vikit Viranuvatti[5]**

A new protocol consisting of 5-fluorouracil (5-FU), methotrexate, and prednisolone (FAP) was selected for the treatment of hepatoma based on the following observations: (*a*) weekly treatment with 5-FU has been as effective as a standard loading dose technique but less toxic (in the treatment of hepatoma, a response rate of 16.7% was achieved [1]); (*b*) the combination of 5-FU and methotrexate with other agents has been effective in treating advanced solid tumors (2); (*c*) methotrexate-resistant cells have shown increasing levels of dihydrofolate reductase and thymidylate synthetase (TS) enzymes (3), and since 5-FU inhibits TS function, it may decrease the TS and possibly reduce some of the resistance of the cells to methotrexate; and (*d*) recent data suggest that adrenal corticosteroids can decrease the influx of cells in late $G_1$ to S phase (4). The FAP combination seems to have more effect on the cells during the $G_1$-S phase transition and the S phase.

## MATERIALS AND METHODS

Eleven patients with histologically proven hepatocellular carcinoma were included in this study. The initial studies included: complete blood counts, platelet counts, or blood smear examinations; chest roentgenogram; liver scan; and routine liver and renal function tests. Blood studies were repeated at least once a week.

The treatment schedule is shown in table 1. The dose of 5-FU was constant during each course of therapy but its administration was delayed if significant granulocytopenia or thrombocytopenia occurred. The patients must have recovered from myelosuppression with a granulocyte count > 1000 cells/mm$^3$, a platelet count > 100,000 cells/mm$^3$, or counts which were adequate on a blood smear. The courses were repeated every 3 weeks and 5-FU plus methotrexate were administered as close to this schedule as possible (at least every 4 weeks). Thus, the ratio of 5-FU to methotrexate (as measured in weeks of treatment) did not have to be 3:1.

The subsequent doses of 5-FU and methotrexate were increased by 10% each if the lowest granulocyte count was ≥ 1500 cells/mm$^3$, the lowest platelet count was ≥ 100,000 cells/mm$^3$, or the counts appeared adequate on a blood smear. The doses were reduced by 10% each if the lowest granulocyte count was < 1000 cells/mm$^3$, and/or the platelet count was < 100,000 cells/mm$^3$, or the platelet count was decreased on a blood smear. Otherwise, there would be no change.

Liver enlargement was measured perpendicularly from the costal margin at the anterior axillary and midclavicular lines and from the tip of the xiphoid cartilage. Other tumor masses were measured as the product of the longest times the widest perpendicular diameters. Complete response (CR) was defined as a complete disappearance of all tumor masses. Partial response (PR) was defined as ≥50% reduction of the sum of all the measurable lesions without evidence of new lesions, and a 30%-49% reduction was classified as a mixed response (MR).

The treatment was stopped when the patient showed relapsing or progressive disease with substantial toxic effects (granulocyte count < 1000 cells/mm$^3$ and/or platelet count < 50,000 cells/mm$^3$ or inadequate counts as determined by blood smear).

## RESPONSE

The clinical characteristics of all patients are shown in table 2. One patient had a CR for 207 days and finally died from progressive disease 441 days

[1]Received June 18, 1975; revised Oct 15, 1975; accepted Oct 22, 1975.

[2]Supported in part by Cyanamid Far East, Thailand.

[3]5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU. Supplied in part by Roche Laboratories, Hoffmann-LaRoche, Inc, Nutley, NJ.

Methotrexate: CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-; amethopterin. Supplied in part by Cyanamid Far East, Thailand.

Prednisolone: CAS reg. No. 50-24-8; pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy-.

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TABLE 1.—FAP protocol: dosage and schedule

| Drugs | Dose schedule | First course: day of treatment 1 2 3 4 5 8 15 | Second course: day of treatment 22 23 24 25 26 29 36 |
|---|---|---|---|
| 5-FU | 300 mg/m²/day | X X X | X* X* X* |
| Methotrexate | 7 mg/m²/day | X X X X X | X X X X X |
| Prednisolone | 25 mg qid | X X X X X | X X X X X |

*Doses were delayed if significant granulocytopenia or thrombocytopenia occurred.

after therapy started. PR was achieved in one patient after two courses of treatment. The duration of remission was 22 months. He died with progressive liver failure from cirrhosis without evidence of progressive malignant disease. His serum α-fetoprotein level decreased from 29,500 to 41 ng/ml. Four patients had MR.

The responses of patients with cirrhotic hepatoma were as good as those without (four responses among seven patients with cirrhotic hepatoma including one PR versus two responses among four without cirrhotic hepatoma including one CR). The three most responsive patients had normal initial liver function tests. No progressive disease occurred in all five patients whose initial liver function tests were not higher than twice the normal level. No responses appeared in four of six patients with higher abnormal initial liver function tests. Two patients with pulmonary metastases did not respond.

## TOXIC EFFECTS

Mild or moderate nausea and vomiting occurred in eight patients. Stomatitis occurred in six patients but only one had severe symptoms. Alopecia was found in three patients. One patient had intermittent diarrhea. Thrombocytopenia occurred in three patients during the first course of therapy. The lowest platelet count was 30,000 cells/mm³ on Day 18 which recovered in 5 days. One patient had hemoperitoneum during the thrombocytopenic period but recovered later. No significant granulocytopenia was found during the first course of treatment.

TABLE 2.—Clinical characteristics of patients with hepatoma treated with FAP protocol

| Patient age (yrs) | Evaluable diseases | Cirrhosis | No. of treatment courses | Response: Type* | Response: Time to response (days) | Response: Duration (days) | Survival time from (days)—: Symptoms | Survival time from (days)—: Diagnosis | Survival time from (days)—: Treatment |
|---|---|---|---|---|---|---|---|---|---|
| 52 | Mesenteric, right supraclavicular lymph nodes | | 13 | CR | 45 | 207 | 625 | 452 | 441 |
| 45 | Liver | + | 22 | PR | 49 | 668 | 763 | 732 | 717 |
| 68 | Liver | − | 2 | MR(47%)† | 34 | 10+ | 102+ | 54+ | 44+ |
| 57 | Liver | + | 2⅓ | MR(40%)† | 14 | 14+ | 104 | 76 | 58 |
| 44 | Liver | + | 1 | MR(39%)† | 12 | 14+ | 161 | 103 | 87 |
| 37 | Liver | + | 1⅓ | MR(31%)† | 21 | 1+ | 124 | 113 | 100 |
| 45 | Liver | + | 2⅔ | NC† | — | — | 107+ | 69+ | 50+ |
| 52 | Liver | − | 3 | FA | — | — | 103 | 94 | 73 |
| 42 | Liver, lungs | + | 3 | FA | — | — | 242 | 97 | 69 |
| 32 | Liver, lungs | − | ⅓ | ED | — | — | 304 | 294 | 5 |
| 43 | Liver | + | ⅓ | ED | — | — | 133 | 81 | 12 |

*FA = failed on an adequate trial (2 or more courses of therapy with progressive disease). ED = early death (within 2 weeks of therapy). NC = no change.

†Maximal response in patients with inadequate followup or lost to followup.

Severe granulocytopenia occurred on Day 16 of the third course of therapy. The infectious complication was responsive to treatment.

## DISCUSSION

A recent review of the results of systemic chemotherapy of hepatoma showed very poor results. Only four of 70 patients responded to various single agents or combination chemotherapeutic programs.[6]

The FAP protocol is an effective treatment leading to PR or CR after two courses of treatment with one long-term survivor. The result of this treatment suggests that this regimen may be more effective in patients who have normal or nearly normal liver function tests and are without pulmonary metastases.

[6]Umsawasdi T. Hepatoma. A challenging disease. Manuscript submitted to the *Journal of the Medical Association of Thailand.*

## ADDENDUM

Death resulting from infection has recently occurred in one patient with severe granulocytopenia during the first course of treatment. It was decided to reduce the doses of 5-FU and methotrexate by 10% from the original protocol.

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# Phase I-II Evaluation of Emetine (NSC-33669) in the Treatment of Epidermoid Bronchogenic Carcinoma[1,2]

**Brief Reports and Preliminary Communications**

**Robert C. Kane, Martin H. Cohen,[3] Lawrence E. Broder, Malcolm I. Bull, Patrick J. Creaven,[4] and Byron E. Fossieck, Jr[5]**

Emetine, an alkaloid salt derived from the root of the plant *Aphaëlis epecacuanha*, has been used principally in the therapy of amebiasis. Biochemically the drug inhibits cellular protein synthesis without affecting DNA or RNA synthesis (1). Screening of this compound for antitumor activity revealed a 72% increase in lifespan (ILS) in murine L1210 leukemia on an every-fourth-day schedule and a 40% ILS in B16 melanoma on an every-3-day schedule.[6]

Phase I clinical trials using an every-fourth-day schedule either iv (2) or sc (3) and a daily sc schedule for 10 days (3) indicated that the principal toxic effects of emetine were reversible muscle weakness and electrocardiographic (ECG) changes. Both toxic and antitumor effects appeared to occur at a total cumulative dose in the range of 15 mg/kg (555 mg/m$^2$) (2). A phase II trial utilizing a daily or twice weekly emetine schedule failed to show an objective tumor response in 16 patients with bronchogenic carcinoma (epidermoid [nine patients], adenocarcinoma [four patients], and anaplastic [three patients]) (4). Street however, has reported tumor response in three patients with epidermoid and three with oat cell bronchogenic carcinoma with emetine at a dose of 1.5 mg/kg weekly iv combined with cyclophosphamide (5).[7] This suggested activity of weekly emetine administration together with the fact that the drug is nonmyelosuppressive prompted us to determine, in patients with epidermoid lung cancer, both the maximally tolerated drug dose on a weekly iv schedule and the frequency of objective tumor response.

## METHODS

Fifteen men (45-65 years of age) with microscopically confirmed epidermoid bronchogenic carcinoma (6) were studied.[8] All of the patients were inoperable and had measurable disease. Three patients had previously undergone thoracotomy, three had received prior radiation therapy, four had received both radiation and chemotherapy, and five were previously untreated. Eight patients had tumor confined to one hemithorax and seven had disseminated disease. All of the patients were ambulatory and had leukocyte and platelet counts > 4500 and > 150,000 cells/mm$^3$ respectively. None of the patients had a second malignant tumor and all gave their informed consent to participate in this study. A history of significant heart disease or an abnormal ECG excluded the patient from the study.

The studies done prior to therapy and repeated weekly included: history and physical examination, body weight, chest radiograph, ECG, complete blood count, blood urea nitrogen, creatinine, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase, calcium, uric acid, creatine phosphokinase (CPK), and caliper measurements of tumor size using the longest and widest perpendicular diameters. ECGs were reviewed weekly before each dose. Standard criteria of tumor response and progression were used (7).

## RESULTS

All of the patients completed at least 4 weeks of therapy and the total dose of emetine ranged from 300 to 675 mg/m$^2$. Four patients were treated with 75 mg/m$^2$ weekly for 4-9 weeks. Only mild toxic effects were noted at this dose level with ECG changes in two patients and elevation of CPK in one. There was no myositis. Toxicity was greater at a weekly emetine dose of 100 mg/m$^2$. In addition to elevations of CPK in three of six patients and the development of sinus tachycardia and/or ST-T wave changes in two, one patient had atrial flutter and another had frequent premature atrial contractions. Clinically significant myositis was also noted in a single patient.

[1]Received July 21, 1975; accepted Oct 22, 1975.

[2]Emetine: CAS reg. No. 316-42-7; emetine, hydrochloride.

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[4]*Present address:* Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, NY.

[5]NCI-VA Medical Oncology Branch, VA Hospital, Washington, DC.

[6]Livingston RB, and Carter SK. Clinical brochure: emetine hydrochloride (NSC-33669). Prepared by the NCI, Bethesda, Md, 1970, 29 pp.

[7]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,-3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[8]Clinical data and therapeutic results presented in tabular form may be obtained by writing to the authors.

Emetine at a dose of 130 mg/m$^2$ weekly produced a spectrum of toxicity similar to that of the lower doses but all the patients developing toxicity had some toxic manifestation by 4 weeks.

The only other toxic effect noted was mild and transient gastrointestinal complaints of nausea and diarrhea. None of the patients experienced chest pain, cardiac dilatation, congestive heart failure, or pain related to the infusion. There were no alterations in leukocyte, platelet, or red blood cell counts, and no alterations in blood biochemistry related to emetine treatment (except that previously mentioned). It was also observed that the serum CPK elevations did not correlate with the ECG changes.

Tumor response was noted in only one patient.[8] In this patient severe toxicity occurred concomitantly with response, preventing any further treatment. In ten patients treatment was discontinued when tumor measurements indicated progressive disease after 4 or 5 weeks of therapy. An additional four patients had stable disease after 4 weeks but progression was documented by 6-9 weeks.

## DISCUSSION

The drug-associated toxicity observed in our study is qualitatively similar to that previously reported (2-4). While the iv route of administration avoids the local discomfort associated with the sc or im route, it does not appear to alter significantly the cumulative dose-related toxicity of emetine.

A moderately toxic dose of emetine suitable for weekly administration is 100-130 mg/m$^2$. This dose produces toxicity similar to a dose of 56 mg/m$^2$ biweekly or 37 mg/m$^2$ daily for 10 days (4).

Although one patient had a 1-month partial tumor response, the patient incurred considerable drug-related toxic effects negating any possible clinical benefit from the treatment. Likewise, in the four patients whose disease was static at 4 weeks but progressive at 6-9 weeks, symptomatic improvement as measured by improved performance status or weight gain did not occur concomitantly. The weekly iv schedule of therapy used in this study does not appear to result in tumor response with an acceptable degree of drug-related toxicity in epidermoid bronchogenic carcinoma.

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# Phase I-II Clinical Trial With Mitoclomine (NSC-114575) in Chronic Lymphocytic Leukemia[1,2,3]

**Brief Reports and Preliminary Communications**

**E. Longeval,[4] D. Gangji, P. Stryckmans, and Y. Kenis[5,6]**

Mitoclomine, a nitrogen mustard molecule bound to a vitamin K derivative (fig 1), was proposed in 1966 by Granger (1) as a useful cytotoxic agent. The basis of this proposal was the demonstration of radiosensitizing properties of synthetic vitamin K (2,3) and vitamin $K_5$ after their concentration in some tumor systems. Granger reports cytotoxic activity of mitoclomine against animal tumors, but in addition it has a rather important depressive effect on lymphoid tissue (1). For example, a single dose in the guinea pig induces a 90% decrease of blood lymphocytes, while there is only a 30% decrease of the total white blood cell count. A similar effect is obtained in the dog when the drug is given orally. Murine investigations performed by Fox et al (4) show a marked depressive activity on both the fixed and the peripheral lymphoid tissues. An immunosuppressive effect of the drug has been shown in other investigations.[7]

Very few studies have been undertaken in man. One incomplete remission among 14 solid tumors and three incomplete remissions among nine lymphomas were reported.[8] In nine patients with solid tumors without bone marrow involvement, eight had a marked decrease of lymphocyte count (85%-99%) without a similar reduction (0-82%) of the polymorphonuclear neutrophil cells (PMN). This observation and the animal studies prompted a phase I-II trial of mitoclomine in chronic lymphocytic leukemia (CLL).

## PATIENTS AND METHODS

Twenty-seven courses of mitoclomine were given to 20 patients (table 1) (11 men and nine women, median age 65 years [range, 49-88 years]) with evolutive CLL who had one of the following features: (*a*) an increase during a 6-month period without therapy of the lymphocyte count from < 20,000 cells/mm$^3$ to at least three times the initial count or from < 30,000 cells/mm$^3$ to at least twice the initial count; (*b*) a lymphocyte level >100,000 cells/mm$^3$; (*c*) a PMN count <2500 cells/mm$^3$; or (*d*) an important and evolutive involvement of the spleen, liver, or lymph nodes.

Mitoclomine was not given to patients with acute hemolysis, severe neutropenia (< 1000 cells/mm$^3$), or thrombocytopenia (< 30,000 cells/mm$^3$) except for one patient who was treated with an initial platelet level of 10,000 cells/mm$^3$. Nine patients had never received any antileukemic therapy before while others had been treated with prednisolone (six patients), chlorambucil (three patients), mechlorethamine (three patients), busulfan (one patient), cyclophosphamide (one patient), or radiotherapy to the spleen (three patients) or lymph nodes (two patients).

Mitoclomine was given orally; the initial daily dosage of each course ranged from 50 mg (one capsule of 100 mg every other day) to 300 mg for testing

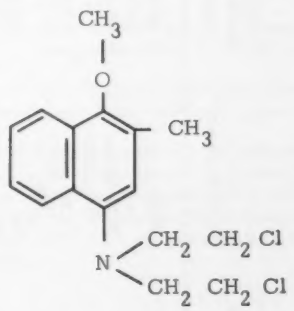


FIGURE 1.—Structure of mitoclomine.

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[2]Supported by grant 3.4559.75 from the Fonds de la Recherche Scientifique Médicale (Belgium) and by the Fonds Cancerologique de la Caisse Générale d'Epargne et de Retraite de Belgique.

[3]Mitoclomine (WBA-7007): CAS reg. No. 17692-54-5; 1-naphthylamine, N,N-bis(2-chloroethyl)-4-methoxy-3-methyl-. Supplied by Ward, Blenkinsop & Co, Ltd, Chemical Manufacturers, Wembley, Middlesex, Great Britain.

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[5]Service de Médecine et Laboratoire d'Investigation Clinique, Institut Jules Bordet, Brussels, Belgium.

[6]We wish to thank Mrs. C. Amez-Droz and Mrs. M. Vaast for their technical assistance.

[7]Hayat M. Personal communication, 1970.

[8]Kenis Y, De Meutter R, Manaster J, et al. Etude préliminaire d'un agent alkylant ayant une action predominante sur les lymphocytes: la mitoclomine. Presented at the Plenary Session of the meeting of the EORTC, Paris, June 20-21, 1969.

TABLE 1.—Data on CLL patients and response to therapy with mitoclomine

| Patient No. | Prior treatment* | Daily dose of mitoclomine (mg/day) | Associated drug | Treatment duration (days) | Total dose (mg) | Lymphocyte count (cells/mm³) | | Nadir PMN count (cells/mm³) | Platelet count (cells/mm³) | | Effects on liver, spleen, or nodes† |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Start | Nadir | | Start | Nadir | |
| 1 | PDN | 50-100 | — | 60 | 5300 | 330,000 | 3500 | 500 | 107,000 | 74,000 | + |
| | — | 50-100 | — | 70 | 5000 | 184,000 | 57,000 | 2500 | 120,000 | 75,000 | + |
| 2 | Mitoclomine, $RT_S$, PDN | 50-200 | PDN | 30 | 2800 | 73,000 | 8500 | 1300 | 49,000 | 46,000 | ? |
| 3 | CPA,PDN, $HN_2$, $RT_S$ | 300 | — | 16 | 4800 | 130,000 | 37,000 | 2000 | 67,000 | 44,000 | + |
| 4 | $HN_2$ | 100-200 | — | 84 | 9800 | 243,000 | 40,000 | 700 | 138,000 | 61,000 | 0 |
| 5 | $RT_S$ | 100-200 | — | 11 | 2000 | 255,000 | 200,000 | 2500 | 111,000 | 35,000 | 0 |
| 6 | — | 100-200 | PDN | 31 | 3800 | 400,000 | 7500 | 1700 | 142,000 | 94,000 | + |
| 7 | $RT_N$, BSF, PDN | 100-200 | — | 50 | 9000 | 245,000 | 10,000 | 200 | 10,000 | 100 | ? |
| 8 | — | 300 | — | 17 | 5100 | 14,000 | 1200 | 800 | 146,000 | 74,000 | ? |
| | Mitoclomine, PDN, $RT_S$ | 100 | — | 15 | 1500 | 130,000 | 2500 | 600 | 108,000 | 98,000 | ? |
| 9 | $RT_N$ | 200-300 | — | 28 | 5500 | 15,000 | 1000 | 400 | 186,000 | 60,000 | + |
| 10 | $RT_S$ | 100 | — | 30 | 3000 | 500,000 | 18,000 | 1700 | 52,000 | ‡ | 0 |
| | $RT_S$, mitoclomine | 100 | — | 18 | 1800 | 180,000 | 21,000 | 2000 | 34,000 | | 0 |
| | $RT_S$, mitoclomine (twice) | 100 | — | 35 | 3500 | 210,000 | 11,000 | 1500 | 30,000 | | 0 |
| 11 | — | 100 | — | 7 | 700 | 108,000 | 45,000 | 3500 | 85,000 | 70,000 | 0 |
| 12 | PDN | 100 | — | 23 | 2300 | 97,000 | 12,000 | 1400 | 169,000 | ‡ | 0 |
| | PDN, mitoclomine | 100 | PDN | 30 | 3000 | 32,000 | 12,000 | 200 | 200,000 | | + |
| 13 | — | 100 | — | 20 | 2000 | 68,000 | 55,000 | 1600 | 177,000 | 124,000 | 0 |
| | Mitoclomine | 100 | — | 14 | 1400 | 32,000 | 16,000 | 1400 | 110,000 | 75,000 | 0 |
| 14 | CAB | 100 | — | 7 | 700 | 675,000 | 670,000 | 30,000 | 109,000 | 10,000 | 0 |
| 15 | — | 200-300 | — | 26 | 4500 | 43,000 | 800 | 500 | 208,000 | 28,000 | + |
| 16 | — | 50-150 | — | 70 | 8500 | 158,000 | 10,000 | 700 | 280,000 | 101,000 | + |
| 17 | — | 50-100 | — | 42 | 2500 | 550,000 | 80,000 | 2400 | 330,000 | 190,000 | + |
| 18 | CAB, PDN | 100 | PDN | 35 | 3500 | 349,000 | 2600 | 600 | 166,000 | 164,000 | + |
| | CAB, PDN, mitoclomine | 100 | — | 25 | 2500 | 180,000 | 19,000 | 1600 | 189,000 | 180,000 | 0 |
| 19 | CAB, PDN, $HN_2$ | 100-300 | — | 50 | 10,150 | 155,000 | 18,000 | 500 | 117,000 | 64,000 | + |
| 20 | — | 100 | — | 36 | 3600 | 76,000 | 2200 | 500 | 288,000 | 23,000 | + |

*PDN = prednisolone; $RT_S$ = radiotherapy to the spleen; $RT_N$ = radiotherapy to the lymph node; $HN_2$ = mechlorethamine; CPA = cyclophosphamide; CAB = chlorambucil; BSF = busulfan.

† + = Diminution of lesions; 0 = no change; ? = not evaluable (absence of evaluable lesions).

‡An increase in the platelet count was observed during therapy.

individual tolerance. This daily dosage was eventually adapted during the course according to side effects, blood toxicity, or therapeutic response. Discontinuation of each course was related to maximal tolerance or therapeutic response which differed widely from patient to patient, so that the duration of the courses varied between 7 and 84 days and the total dose of mitoclomine for each course varied between 700 and 10,150 mg. The drug was given alone in 23 courses and combined with prednisolone[9] (40-60 mg daily) in four courses. Because most patients were outpatients, blood counts and measurements of tumors were performed once a week (± 3 days).

## RESULTS

Table 1 indicates that the blood lymphocyte count decreased during all the courses and fell to < 20% of the initial level in 19 courses. The median initial level was 157,000 cells/mm$^3$ (ranging between 14,000 and 675,000 cells/mm$^3$) and the median nadir was 12,000 cells/mm$^3$ (ranging between 800 and 670,000 cells/mm$^3$). Usually, a decrease of lymphocytosis appeared within the first week of treatment and the nadir developed within 0-2 weeks after discontinuation of the drug. In the four courses (patient Nos. 5, 13, and 14) where blood lymphocytes fell by < 50% of the initial count, mitoclomine had to be interrupted because of thrombocytopenia. It is noteworthy that the patients (Nos. 8, 10, and 13) who received two or three courses of mitoclomine had a similar response to each course. A clear relationship exists between the lymphocyte response and the reduction of organ infiltration (lymph nodes, liver, spleen). A persisting drug effect, as defined by a blood lymphocyte plateau (nadir ± 15%) for at least 1 month after discontinuation of the drug, was noted in ten patients. In one patient, this persisting effect lasted for 16 months.

The platelet level decreased by more than 50% of the initial level in only nine courses and rose slightly in five. Overall, the toxicity to the platelets was statistically less significant than the lymphocyte depletion ($P < 0.05$). This trend also seems to be present with regard to the toxicity to PMN, but these counts fell below a dangerous level of 1000 cells/mm$^3$ in 12 courses out of 27. Hemoglobin levels increased in seven courses and decreased by > 20% of the initial level in eight courses. The combination of mitoclomine and prednisolone did not lead to striking results with regard to toxicity or therapeutic response.

In half of the courses treatment was well tolerated; nausea and moderate vomiting occurred in 12 courses and diarrhea in three. Discontinuation of the drug was related to those gastrointestinal side effects in five cases, to hematologic depression in ten cases, to satisfactory blood response in seven cases, and to extra-medical reasons in five cases. No alopecia was noted. Due to the variability of the clinical aspects of the disease, the small number of patients in our series, and the administration of other treatments after mitoclomine in many of them, the influence of mitoclomine on survival could not be investigated.

## CONCLUSION

This phase I-II study shows that mitoclomine is an active agent in reducing both blood lymphocytosis and organ infiltration in CLL. The neutropenic effect seems to be less important than the lymphopenic effect. Nevertheless, severe neutropenia which was observed in half of the patients represents the greatest threat in the administration of mitoclomine. Gastrointestinal side effects are tolerable in most patients and no alopecia is noted. When mitoclomine is given as a continuous treatment the daily dose of 100 mg seems sufficient to obtain a good therapeutic response. It should be worthwhile to test an intermittent dosage schedule, which was proven useful with chlorambucil (5). We propose to compare mitoclomine with other agents active in CLL in terms of therapeutic effectiveness and cross resistance.

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[9]Prednisolone: NSC-9900; CAS reg. No. 50-24-8; pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy-.

# 5-Day Iv Infusion With 5-Fluorouracil (5-FU; NSC-19893) for Gastroenteric Carcinoma After Failure on Weekly 5-FU Therapy[1,2,3]

**Brief Reports and Preliminary Communications**

**Gilbert J. Hum[4] and Joseph R. Bateman[5]**

Until recently the administration of 5-fluorouracil (5-FU) by iv infusion has not been shown to be therapeutically superior to rapid injection of the drug (1-4). Previous reports described the observations for infusion periods of up to 8 hours. Using a 24-hour infusion of 5-FU at doses of 15 mg/kg/day to as high as 40 mg/kg/day for periods ranging from 4½ days to as long as 36 days, Sullivan et al (5) reported toxicity was reduced by a factor of two compared with a single daily rapid iv administration. No response data were given.

Interest in prolonged continuous 5-FU infusion has been revived by recent reports of superior results of 5-day continuous iv infusion over bolus injection. The abstract reports do not indicate whether the patients had received prior 5-FU therapy (6-8) and none of the patients had prior chemotherapy in a study done by Seifert et al (9). Our study suggests that a 5-day continuous infusion of 5-FU does not have clinical usefulness for patients who previously failed to respond to 5-FU therapy administered once weekly.

## MATERIALS AND METHODS

All patients had histologically confirmed adenocarcinoma of gastroenteric origin with measurable tumor parameters and a Karnofsky performance score of ≥ 50 (10). The required minimal entry measurement limits were: white blood cell (wbc) count ≥ 4000 cells/mm$^3$, platelet count ≥100,000 cells/mm$^3$, total bilirubin level ≤ 2 mg/100 ml, and creatinine level ≤ 2 mg/100 ml. All patients were previously treated with 5-FU given weekly iv or orally. Three of these patients received concomitant warfarin and one patient received concomitant cyclophosphamide.[6] Two patients also had previous radiotherapy.

Unless resolved by the time of entry, the following conditions prevented entry into the study: intestinal obstruction, active gastrointestinal bleeding (≥ 3+ grade occult blood in stool on two of three separate specimens), stomatitis, or unexplained diarrhea (three times per 24-hour period). Complete blood and platelet counts were done daily during the 5 days of 5-FU administration and weekly during the 3-week interval rest periods.

If after a course of therapy the wbc count was < 3000 cells/mm$^3$ and/or the platelet count was < 75,000 cells/mm$^3$ by the next scheduled treatment day, 5-FU was delayed until the wbc and platelet counts had recovered to ≥4000 and ≥100,000 cells/mm$^3$ respectively before treatment was resumed. In addition, treatment was discontinued until resolution of any punctate ulcerations of the gums, buccal mucosa, or palate, unexplained diarrhea (three times per 24-hour period), or epistaxis. Patients were given a minimum of two treatment courses.

5-FU was given at a dose of 30 mg/kg/day for 5 days as a continuous iv infusion in 5% dextrose and water. An interval rest period of at least 3 weeks and 2 days separated each course of treatment.

## RESULTS AND DISCUSSION

Fourteen patients were entered in the study and all were evaluable for response. There were seven patients with colorectal primary tumors, four with tumors of gastric origin, one with a tumor of pancreatic origin, and two whose metastatic adenocarcinoma was of uncertain but suspected gastrointestinal origin. One patient completed eight courses, two completed five, four completed four, four completed three, one completed one, and two completed less than one. The three patients who completed less than two full courses did so because of lack of cooperation on the part of one patient and the early death of the other two patients (Days 2 and 3 of

[1]Received Aug 18, 1975; accepted Oct 22, 1975.

[2]Supported in part by Public Health Service grant CA-05186 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]5-Fluorouracil: CAS reg. No. 51-21-8. Supplied by Drug Research and Development Program, Division of Cancer Treatment, NCI, for iv use and purchased from commercial sources for oral use.

[4]*Reprint requests to:* Gilbert J. Hum, MD, Medical Oncology Division at John Wesley County Hospital, University of Southern California, 2825 S Hope St, Los Angeles, Calif 90007.

[5]University of Southern California, Los Angeles.

[6]Warfarin: NSC-59813; CAS reg. No. 81-81-2; coumarin, 3-(α-acetonylbenzyl)-4-hydroxy-; Coumadin.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

TABLE 1.—Hematologic and gastrointestinal toxicity: 5-day iv infusion with 5-FU

| Toxic effects | No. of patients with grade—* | | |
|---|---|---|---|
| | Minimal | Moderate | Severe |
| Gastrointestinal | | | |
| Nausea and/or vomiting | 6 | 4 | 0 |
| Diarrhea | 3 | 1 | 0 |
| Stomatitis | 2 | 4 | 0 |
| Hematologic | | | |
| Leukopenia | 1 | 1 | 2 |
| Thrombocytopenia | 3 | 0 | 0 |

*Gastrointestinal: *Minimal* = 1-2 emesis or stool/day; superficial mucosal ulceration without secondary infection. *Moderate* = 3-5 emesis or stool/day; mucosal ulceration with secondary infection (controllable with antibiotics). *Severe* = > 5 emesis or stool/day (persistent and uncontrolled with antiemetics or antidiarrhea drugs); mucosal ulceration with no systemic infection but alimentation not possible.

Hematologic (cells/mm³): *Minimal* = wbc 3000-3999, platelets 100,000-147,999. *Moderate* = wbc 2000-2999, platelets 60,000-99,999. *Severe* = wbc 1000-1999, platelets 35,000-59,999.

course No. 1). No objective responses (> 50% tumor shrinkage with subjective improvement for ≥ 1 month) were observed. In one patient with adenocarcinoma of gastric origin no disease progression with subjective improvement for 7+ months continues, and in two other patients with adenocarcinoma of the colon no disease progression without subjective improvement occurred (lasting 3 months in one patient and 6 months in the other).

The major gastrointestinal toxic effects were nausea or vomiting (ten of 14 patients), stomatitis (six of 14 patients), and diarrhea (four patients) (table 1). However, the diarrhea and nausea and vomiting were not dose limiting whereas stomatitis was the cause for reduction of a course of 5-FU infusion to <5 days and/or the cause for delay of subsequent scheduled courses in two patients. The initial stomatitis occurred as early as the third day of the first course of 5-FU infusion and as late as the completion of the fourth course. All instances of nausea and vomiting or diarrhea responded to antiemetics or antidiarrhea medications.

Hematologic toxic effects were minimal (table 1). Leukopenia was observed in four patients but was the cause of a prolonged interval rest period in only one patient. For this patient course No. 4 was delayed for 4 weeks because the nadir leukocyte count was 1800 cells/mm³ during course No. 3; complete leukocyte recovery did not occur until 17 weeks after completion of the fourth course. The patient was an uncooperative narcotic addict who frequently missed the scheduled return visits. Thrombocytopenia of a minimal degree was seen in only three patients. Severe leukopenia occurred in one patient after the first course of treatment and in a second patient after five courses; full recovery was seen on Days 10 and 7.

A peculiar persistent hyperpigmentation limited to the skin over the upper extremity veins was observed in two patients, occurring after five courses in one patient and after six courses in the other. Side effects which were less common included alopecia and anorexia.

The administration of 5-FU by 5-day infusion appears to have little clinical usefulness for patients who have been previously treated with 5-FU. Each of our 14 patients had shown no objective response to prior trials of at least six weekly doses of 5-FU. Twelve patients had received an iv bolus injection and two had received the drug orally. Our data confirm other reports of minimal hematologic toxicity when 5-FU is given as a 5-day constant infusion. Mucosal and gastrointestinal toxic effects were the most frequently observed side effects. There were no drug-related deaths.

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# Phase II Study of Adriamycin (NSC-123127) in Patients With Metastatic Melanoma[1,2,3]

**Brief Reports and Preliminary Communications**

**William J. Sieper[4], Michael J. Mastrangelo[5] and Robert E. Bellet[5,6]**

Adriamycin is an anthracycline antibiotic isolated from the broth of *Streptomyces peucetius* var. *caesius*. This agent showed significant activity against several animal tumors in preclinical studies (1) and was therefore chosen for human investigation. A recent review (1) describes adriamycin as having significant antitumor activity in human sarcomas and breast and bladder carcinomas. As a result of its broad spectrum of activity in human malignancy, a phase II evaluation of adriamycin in metastatic malignant melanoma was undertaken.

## MATERIALS AND METHODS

Adriamycin was administered to 22 patients with advanced measurable metastatic malignant melanoma. Histologic confirmation of metastatic melanoma was required for entry into the study. Patients with a life expectancy of < 3 months, prior major pelvic radiation therapy (> 2000 rads), or systemic chemotherapy within 28 days of the start of this study were ineligible for treatment. Patients with a hemoglobin level < 9 g/100 ml, a white blood cell (wbc) count < 4000 cells/mm$^3$, a platelet count < 100,000 platelets/mm$^3$, or a blood urea nitrogen (BUN) level > 25 mg/100 ml were also ineligible. Patients with congestive heart failure, cardiomegaly, or an abnormal electrocardiogram (ECG) were excluded from the study.

The patients included 15 men and seven women. The mean age for men was 50 years (range, 23-76 years) and the mean age for women was 55 years (range, 42-69 years). All patients had received prior sequential systemic chemotherapy with DTIC and BCNU plus vincristine. One woman receiving DTIC therapy had achieved a partial remission (PR) of 23 months' duration. Another woman had PR for 5 months and one man had a complete response for 17 months after receiving BCNU plus vincristine therapy.

Adriamycin was administered at a dose of 60 mg/m$^2$ iv push to 16 patients. Six patients with hepatic metastatic disease or limited bone marrow reserve received adriamycin at a dose of 40 mg/m$^2$. The courses of therapy were repeated at 21-day intervals until a measurable progression (> 25% increase in tumor volume) was shown. Palpable dermal, subcutaneous, and lymph node metastases were measured every 3 weeks. Pulmonary parenchymal masses were evaluated with chest roentgenograms and hepatic metastatic lesions were evaluated with liver scans at 3-week intervals.

Hemoglobin determinations and wbc and platelet counts were performed on Days 1, 14, and 21 of each treatment cycle. BUN, total bilirubin, serum glutamic oxaloacetic transaminase, alkaline phosphatase, creatine phosphokinase (CPK), chest roentgenogram, and ECG were performed initially and at 21-day intervals.

Adriamycin was supplied in sterile vials containing 10 mg of red, lyophilized powder. Each vial of drug was reconstituted with 5 ml of sterile water immediately before the iv injection.

## RESULTS

Of the 22 patients treated with adriamycin, 19 were evaluable for toxicity and response. Three patients died of metastatic melanoma within 21 days after therapy started and were therefore considered unevaluable.

## TOXICITY

Five patients had leukopenia of < 2000 cells/mm$^3$ and five patients had thrombocytopenia of < 100,000 platelets/mm$^3$. Two patients had significant anemia on the basis of bone marrow suppression. In most patients acute gastrointestinal toxic effects consisting of nausea, emesis, and diarrhea continued for 1-2 days.

Five patients had acute cardiotoxicity manifested by transient ECG abnormalities. All three patients

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[3]Adriamycin: CAS reg. No. 23214-92-8. Supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI.

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who had sinus tachycardia had concurrent transient elevations in serum CPK values. Two additional patients with transient elevations of CPK values did not manifest ECG changes. No patient showed evidence of cardiomyopathy. Alopecia and stomatitis were noted. No other toxic side effects were seen.

## RESPONSE

No patient had objective improvement during therapy with adriamycin. All of the patients manifested rapidly progressive and often explosively progressive disease in spite of the treatment. Of 19 evaluable patients eight had rapid progression of disease after one course of adriamycin, nine progressed after two courses, and two received three courses prior to progression.

## DISCUSSION

Successful chemotherapeutic management of patients with metastatic malignant melanoma is a difficult problem. DTIC and the nitrosoureas are the only cancer chemotherapeutic agents with consistent antimelanoma activity. The demonstration of a broad spectrum of activity against a host of human malignancies qualified adriamycin for a phase II evaluation in patients with metastatic melanoma who had been previously treated with DTIC and BCNU plus vincristine.

In 19 previously treated patients with metastatic melanoma who received adriamycin in therapeutic doses there were no objective remissions. In fact, there was no suggestion of disease stabilization. The two patients who received three courses of adriamycin had slowly progressive disease before therapy which continued at the same rate during therapy. Although all patients had advanced disease and significant total body tumor burdens, > 80% had measurable dermal, subcutaneous, or nodal metastases, and therefore were considered reasonable candidates for response. In addition, three patients (16%) had previously responded to either DTIC or BCNU plus vincristine systemic chemotherapy so that this group of patients could not be considered chemotherapy resistant. Our data are consistent with the report of O'Bryan et al (2) that in 13 patients with advanced malignant melanoma no responses to adriamycin were noted.

Adriamycin toxic effects which were experienced by our patients are consistent with those compiled by Blum and Carter (1). Although 26% of our patients had transient ECG changes, no patient had evidence of congestive heart failure or cardiomyopathy. No patient, however, received a total dose of adriamycin > 180 mg/m$^2$ which is well below the 550 mg/m$^2$ limit.

## CONCLUSION

Adriamycin administered at the standard dose of 60 mg/m$^2$ iv every 21 days is ineffective in the treatment of advanced metastatic malignant melanoma. This lack of antitumor activity does not suggest that adriamycin be given a trial in the treatment of patients with melanoma at an earlier stage in their metastatic disease.

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# ANNOUNCEMENTS

## Symposium on "The Non-Hodgkin's Lymphomas"

***San Francisco, California, September 30-October 2, 1976***

The symposium on "Non-Hodgkin's Lymphomas" to be held on September 30-October 2, 1976, at the San Francisco Hilton Hotel in San Francisco, California will be sponsored by the Cancer Clinical Investigation Review Committee of the National Cancer Institute.

*For further information write:* Stephen E. Jones, MD, Department of Medicine, University of Arizona Medical Center, Tucson, Ariz. 85724.

## Oncology Placement Service

The American Society of Clinical Oncology's Clearing House of Oncologic Opportunities is accepting inquiries from applicants concerning staff positions and oncology fellowships, as well as announcements of openings for such applicants. The service is open to members and non-members of ASCO. Please direct all inquiries and announcements to Michael J. Brennan, MD, American Society of Clinical Oncology, 110 E Warren, Detroit, Mich 48201.

## Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigation

***Sheraton Biltmore, Atlanta, Georgia, February 26-28, 1976***

This Symposium is sponsored by the Clinical Investigations Branch and the Cancer Clinical Investigation Review Committee of the National Cancer Institute. The program will include discussion of: Animal Models and Screening; Pharmacodynamics; Neuropathology; Multimodal Therapy and Unusual Methods of Treatment; Cooperative Clinical Trials; and Criteria of Evaluation and Problems of Analysis.

The Program Committee: *Audrey E. Evans*, Children's Hospital of Philadelphia, Pa; *Cornelia Dettmer*, Christ Hospital, Cincinnati, Ohio, *Michael D. Walker*, Baltimore Cancer Research Center, Md; *Charles Wilson*, University of California at San Francisco; and *Giulio J. D'Angio*, Memorial Sloan-Kettering Cancer Center, New York, NY, *Chairman*.

This symposium is open to members of the medical and allied health professions. Advance registration requested, no registration fee.

*For information write to:* Audrey E. Evans, MD, The Children's Hospital of Philadelphia, One Children's Center 3400 Civic Center Blvd, Philadelphia, Pa 19104.

## 67th Annual Meeting of the American Association for Cancer Research, Inc

***Toronto, Canada, May 6-8, 1976***

The scientific sessions of the 67th Annual Meeting of the American Association for Cancer Research, Inc, will be held at the Four Seasons-Sheraton, Toronto, Canada, from 8:30 AM, Thursday, May 6, until 5:00 PM, Saturday, May 8, 1976. Incorporated into this meeting will be a Symposium on "Environmental Carcinogenesis" being arranged by the National Cancer Institute of Canada for the Biennial Meeting of the International Union Against Cancer and representing the 11th Canadian Cancer Research Conference. The meeting of the Association will be preceded by that of the American Society of Clinical Oncology, to be held at the immediately adjacent Hotel Toronto on May 4 and 5, 1976.

Registration for the Association meeting will be conducted in the afternoons and evenings of May 4 and 5 and throughout the course of the meeting. Nonmembers may obtain programs and purchase abstracts of the papers at the time of registration. By action of the Board of Directors, a registration fee of $15.00 will be charged each member and nonmember attending the scientific sessions; a special rate of $5.00 will be charged predoctoral students who present a statement, signed by the registrar, dean, or department head of their university or college, confirming their status. Admission to the scientific sessions will be restricted to properly registered persons.

A copy of the detailed regulations involving submission of abstracts and presentation of papers have been sent to each active member of the Association as part of the customary Annual Letter which was issued in October 1975. Corresponding members and nonmembers may obtain copies of the regulations and hotel reservation cards from the Secretary-Treasurer. Abstracts that are submitted in conformity with the regulations and are considered of acceptable quality will be published in the March 1976 *Proceedings of the American Association for Cancer Research*, which will be mailed by the publisher to all paid-up subscribers to the journal *Cancer Research*.

*For further information write:* Hugh J. Creech, Secretary-Treasurer, American Association for Cancer Research, The Institute for Cancer Research, Fox Chase, Philadelphia, Pa 19111.

## Hematology/Oncology Fellowship

Hematology/Oncology fellowship applications are now being accepted for a 2-year fellowship training program at the Veterans' Administration Hospital in Long Beach, California.

This program offers opportunities in clinical or laboratory research. The hospital is a member of the Western Cancer Study Group and contributes to the National Institutes of Health's cooperative cancer chemotherapy programs. The section has 20 beds and active outpatient clinics. Present activities include research in cell cycle kinetics in myeloma and DNA metabolism. One or 2 years of medical residency is a prerequisite.

Individuals interested in a fellowship beginning July 1, 1976, are invited to submit a letter and curriculum vitae to: Dr. J. Brook, Chief, Hematology/Oncology Section, VA Hospital, 5901 E 7th St, Long Beach, Calif 90801.

## Symposium on Recent Advances in Cancer Treatment Research

### Baltimore, Maryland, March 25-27, 1976

This symposium will be Codirected by Dr. Theodore Woodward, Professor and Chairman, Department of Medicine, University of Maryland, and Dr. Peter Wiernik, Chief, Clinical Branch, Baltimore Cancer Research Center. A broad course focus will cover such topics as lung tumors, soft tissue sarcomas, Hodgkin's disease, and many other areas with internationally renowned speakers. This course is being tailored for the general physician as well as practicing or research-oriented oncologists. Since 1976 is the Bicentennial year, this as well as many of our other programs will also include a special planned activity (eg, tour, special teaching session, etc).

*For further information please contact:* Mitchell Perlin, Course/Media Coordinator, Program of Continuing Education, School of Medicine, University of Maryland, Baltimore, Md 21201.

## CORRECTION

The authors of the article "Tabular Analysis of the Chemotherapy of Solid Tumors" which appeared in the October issue of *Cancer Chemotherapy Reports* Part 3 (Vol. 6, No. 2) would like to point out an error which appeared in the section entitled Colon Cancer. The fourth sentence in that section should read as follows: "Methotrexate is the *only other* antimetabolite showing some hint of activity and is now undergoing confirmatory study." They wish to make this change since obviously 5-fluorouracil, which is an antimetabolite, is also active in colon cancer.

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